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EPA METHOD STUDY 26, METHOD 613, 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN

by

Fred D. Hileman, David E. Kirk, Thomas Mazer, Arthur D. Snyder,
Beverly J. Warner and Carl R. McMillin
Monsanto Research Corporation
Dayton, Ohio 45407

Contract 68-03-2856

Project Officer

Raymond Wesselman
Quality Assurance Branch
Environmental Monitoring and Support Laboratory
Cincinnati, Ohio 45268

ENVIRONMENTAL MONITORING AND SUPPORT LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
Cincinnati, Ohio 45268

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The experimental design and results of an interlaboratory study for dioxin in water is described herein. The interlaboratory study of EPA Method 613 consisted of the replicate analyses of a performance evaluation sample used primarily for determining laboratory competence and subsequent analyses of six sample concentrations and a blank in six different waters (42 samples). Statistical analyses and conclusions in this report are based on analytical data obtained by eleven collaborating laboratories.

Participating laboratories were selected based upon technical evaluation of proposals and upon the analytical results of the prestudy samples. Data obtained from the interlaboratory study were analyzed employing US EPA's series of computer programs known as the Interlaboratory Method Validation Study (IMVS) system, which implements the concepts recommended in ASTM Standard D 2777. The statistical analyses included tests for the rejection of outliers, estimation of mean recovery (accuracy), estimation of single-analyst and overall precisions, and tests for the effect of water type on accuracy and precision.

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FOREWORD

Environmental measurements are required to determine the quality of ambient waters and the character of waste effluents. The Environmental Monitoring and Support Laboratory (EMSL)-Cincinnati conducts research to:

- Develop and evaluate techniques to measure the presence and concentration of physical, chemical, and radiological pollutants in water, wastewater, bottom sediments, and solid waste.
- Investigate methods for the concentration, recovery, and identification of viruses, bacteria, and other microorganisms in water.
- Conduct studies to determine the responses of aquatic organisms to water quality.
- Conduct an Agency-wide quality assurance program to assure standardization and quality control of systems for monitoring water and wastewater.

This publication, EPA Method Study 26, Method 613, 2,3,7,8-Tetrachlorodibenzo-p-dioxin, reports the results of EPA's interlaboratory method study for analysis of the 2,3,7,8-TCDD isomer.

Federal agencies, states, municipalities, universities, private laboratories, and industry should find this evaluative study useful in monitoring and controlling pollution in the environment.

Robert L. Booth

Robert L. Booth, Acting Director
Environmental Monitoring & Support Laboratory

ABSTRACT

The experimental design and results of an interlaboratory study for an analytical method to detect 2,3,7,8-tetrachlorodibenzo-p-dioxin in water are described herein. In EPA Method 613, water containing an internal standard (labeled 2,3,7,8-TCDD) is extracted with methylene chloride, concentrated, exchanged to hexane, and then subjected to capillary column gas chromatography/mass spectrometric (GC/MS) analyses, which allow for separation and measurement of the 2,3,7,8-TCDD isomer in the extract. The method study consisted of the replicate analyses of a performance evaluation sample used primarily for determining laboratory competence for 2,3,7,8-TCDD specific analyses and subsequent analyses of six sample concentrations and a blank in six different waters (42 samples). Statistical analyses and conclusions in this report are based on analytical data obtained by eleven collaborating laboratories.

Participating laboratories were selected based upon technical evaluation of proposals and upon the analytical results of pre-study samples. Data obtained from the interlaboratory study were analyzed employing USEPA's series of computer programs known as the Interlaboratory Method Validation Study (IMVS) system, which implements the concepts recommended in ASTM Standard D 2777. The statistical analyses included tests for the rejection of outliers, estimation of mean recovery (accuracy), estimation of single-analyst and overall precisions, and tests for the effects of water type on accuracy and precision.

This report was submitted in fulfillment of contract 68-03-2856 by Monsanto Research Corporation under the sponsorship of the U.S. Environmental Protection Agency and covers a period from November 1979 to December 1982.

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SECTION 1

INTRODUCTION

The analytical laboratories of the U.S. Environmental Protection Agency (EPA) gather water quality data to provide information on water resources, to assist research activities, and to evaluate pollution abatement activities. The success of these pollution control activities, particularly when legal action is involved, depends upon the reliability of the data provided by the laboratories.

Under provisions of the Clean Water Act, the EPA is required to promulgate guidelines establishing test procedures for the analysis of pollutants. The Clean Water Act Amendments of 1977 emphasize the control of toxic pollutants and declare the 65 "priority" pollutants and classes of pollutants to be toxic under Section 307(a) of the Act. This report is one of a series that investigates the analytical behavior of selected priority pollutants and suggests a suitable test procedure for their measurement. The priority pollutant to be analyzed by Method 613 is 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD).

The Environmental Monitoring and Support Laboratory-Cincinnati (EMSL) of the EPA develops analytical methods and conducts a quality assurance program for the water laboratories to maximize the reliability and legal defensibility of water quality information collected by EPA laboratories. The responsibility for quality assurance (QA) is assigned to the Quality Assurance Branch (QAB). One of the QA activities is to conduct interlaboratory tests of

the methods in order to generate precision and accuracy data. This study reports the results of the interlaboratory study on Method 613 (Study 26).

The method evaluated in this study was prepared by the Physical and Chemical Methods Branch of EMSL-Ci and is a revision of the method described in the Federal Register on December 3, 1979 [1]. The revised method is reproduced in Appendix A of this report.

Phase I included preliminary method study work conducted by Monsanto Research Corporation (MRC) to (1) evaluate the original Method 613 as published in the December 3, 1979 Federal Register [1]; (2) determine the minimum detection limit of the method; and (3) determine the storage stability of 2,3,7,8-TCDD/acetone solutions in ampuls over a 90-day period. As a result of the Phase I study, Method 613 was revised to substantially alter the sample processing steps. These revisions are now included in the version of Method 613 as presented in Appendix A.

Phase II involved the selection of participating laboratories that could demonstrate satisfactory analytical results using their personnel and equipment. This selection was based partially on analysis of a performance evaluation sample of mixed tetrachlorodibenzo-p-dioxin isomers for 2,3,7,8-TCDD.

Phase III, the formal interlaboratory study, required the analysis of 2,3,7,8-TCDD in six water types at each of six concentrations (three Youden pairs) in addition to the analysis of all water blanks with no spiked compound. Each participating laboratory then forwarded a report to Monsanto Research Corporation containing all data obtained, and a completed questionnaire covering specifics on the analysis including the source of distilled, tap and surface waters, instrumentation used, GC conditions, and specific problems encountered in the analyses.

The final step in the study was a statistical analysis of all data by Battelle Columbus Laboratories, Columbus, Ohio, under EPA contract 68-03-2624 employing USEPA's system of computer programs known as the Interlaboratory Method Validation Study (IMVS) system.

SECTION 2

CONCLUSIONS

The objective of this study was to characterize the performance of Method 613 in terms of accuracy, overall precision, single-analyst precision, and the effects of water types on accuracy and precision. Through statistical analyses of 396 analytical values, estimates of accuracy and precision were made; these are expressed as regression equations in Table 1.

The accuracy is obtained by comparing the mean recovery to the true values of the concentration. The average percent recovery is 91% with a range from 86% to 96%.

The overall relative standard deviation (RSD) indicates the precision associated with measurements generated by a group of laboratories. The average percent relative standard deviation is 21% with a range of 18% to 23%.

The relative single-analyst standard deviation (RSD-SA) indicates the precision associated within a single laboratory relative to the mean recovery. The average percent relative standard deviation for single analyst is 16% with a range of 13% to 18%.

A statistical comparision of the effect of the water types indicated no statistically significant differences among water types.

Correct activation of the alumina column is critical. Overactivation causes adsorption which results in incomplete or no elution of TCDD from the column.

TABLE 1. REGRESSION EQUATIONS FOR ACCURACY AND PRECISION 2,3,7,8-TCDD (Concentration Range 21-202 ppt)

Water type	2,3,7,8-TCDD
Distilled water	
Single-analyst precision	SR = 0.13X + 1.29
Overall precision	S = 0.19X + 0.28
Accuracy	$\bar{X}$ = 0.86C + 1.45
Tap Water	
Single-analyst precision	SR = 0.01X + 7.78
Overall precision	S = 0.22X + 0.80
Accuracy	$\bar{X}$ = 0.81C + 4.82
Surface Water	
Single-analyst precision	SR = 0.09X + 2.25
Overall precision	S = 0.21X - 1.11
Accuracy	$\bar{X}$ = 0.80C + 3.93
Wastewater 1	
Single-analyst precision	SR = 0.25X - 4.41
Overall precision	S = 0.28X - 4.63
Accuracy	$\bar{X}$ = 0.94C + 1.03
Wastewater 2	
Single-analyst precision	SR = 0.06X + 2.71
Overall precision	S = 0.16X + 3.12
Accuracy	$\bar{X}$ = 0.82C + 4.30
Wastewater 3	
Single-analyst precision	SR = 0.16X + 0.49
Overall precision	S = 0.20X + 0.01
Accuracy	$\bar{X}$ = 0.78C + 4.26

$\bar{X}$ = mean recovery
C = true concentration

Some laboratories experienced poor recovery and poor precision on the labeled compounds. As a quality control measure, a second independent internal standard or surrogate should be used to detect possible error in known concentration, evaporated concentration or incomplete dissolution of standards.

Six of the eleven laboratories experienced little or no difficulty with Method 613. In these cases, over 98% of the data points were retained (4 outliers out of 216 analyses). All data from one laboratory were rejected since Method 613 was not followed in that an incorrect internal standard was employed. Of the remaining four laboratories, 66% of the data were found to be outliers. In these cases, the following three problems were evident:

(1) difficulty with alumina cleanup (Laboratories 4 and 11).
(2) no recovery on C^{13}_{12} internal standard (Laboratory 6)
(3) lack of sensitivity at low levels of TCDD (Laboratory 8).

It is concluded that the higher than expected rejection of data from five laboratories was due to inexperence of the laboratories with the method and the lack of specific direction on the alumina activation.

SECTION 3

RECOMMENDATIONS

Method 613 is recommended for the analysis of 2,3,7,8-TCDD in municipal and industrial discharges. The method yields satisfactory accuracy and precision.

To increase sensitivity place the end of the column as close to the ion source as possible.

It is recommended that the method be changed to clarify the alumina column cleanup procedure. The alumina column should be checked for proper activity. Overactivated alumina can cause adsorption of 2,3,7,8-TCDD which is not eluted or incompletely eluted by the solvent eluent.

Because some laboratories reported poor precision and experienced interferences with the labeled compounds, the use of a second internal standard or surrogate is recommended to detect errors in known concentration, evaporated concentrations or incomplete dissolution of standards.

SECTION 4

DESCRIPTION OF STUDY

SELECTION OF PARTICIPATING LABORATORIES

Requests for proposals for the Method 613 Interlaboratory Method Study were sent to over 250 potential laboratories. The participating laboratories would be required to conduct capillary column GC/MS analysis of 2,3,7,8-TCDD, using a capillary column which at that time was available only in glass (Silar-10C). When fewer than the desired number of responses were received, it was determined that the reasons were primarily a lack of capillary GC/MS capability or a concern about the safety and handling of 2,3,7,8-TCDD.

Changes were made in Method 613 to make sample preparation easier to perform. It was decided that Monsanto Research Corporation would supply the dioxin standards so that high hazard facilities would not be required by the participating laboratories. Also, all of the wastes generated during the study would be returned to MRC for disposal. These changes made it possible for twenty-nine laboratories to respond to a request for proposals. The responses were ranked technically based on experience, facilities, quality assurance/quality control, and personnel. Arrangements were made with eighteen laboratories that ranked high technically to participate in the method study contingent upon acceptable analysis of performance evaluation (PE) samples. Two additional laboratories received the PE samples for analysis by Method 613 as a quality assurance check on other projects. Their data are included in the interlaboratory statistics for the PE samples.

PERFORMANCE EVALUATION SAMPLES

The objectives of the PE study were to (1) determine if the laboratories could separate the 2,3,7,8-TCDD from the other potentially interfering isomers; and (2) determine if the laboratories methodology had adequate sensitivity for the study. The laboratories were requested to spike four one-liter samples of water from one vial of spiking solution and analyze the samples using Method 613 with the alumina column cleanup.

At the time the PE samples were prepared, only Silar-10C had been shown to give resolution of 2,3,7,8-TCDD from the other 21 tetrachlorodibenzo-p-dioxin isomers [2]. EPA Method 613 specified that if a positive peak is found, it must be confirmed using a Silar-10C column or its equivalent. A PE sample was synthesized that included approximately 50 parts per trillion (ppt) of the 2,3,7,8-, 1,2,3,4-, and 1,4,7,8-TCDD isomers. The latter two isomers elute immediately before and immediately after the 2,3,7,8-TCDD on Silar-10C [2] and are therefore the most difficult isomers to separate from 2,3,7,8-TCDD with this column.

Prior to sending the PE samples to the participating laboratories, Monsanto Research Corporation evaluated a 60-m SP-2330 glass capillary column (Supelco, Bellefonte, Pennsylvania) for its applicability to the separation of the TCDD isomers since SP-2330 is a highly polar liquid phase with McReynolds constants very close to those of Silar-10C. The SP-2330 column was found to give superior chromatographic resolution and peak shape compared to that obtained with the Silar-10C column.

Performance evaluation kits were sent to the 20 laboratories in September, 1981. The following were provided by Monsanto Research Corporation:

- Sample chromatogram of the PE mix.
- Instructions for PE sample analysis including handling procedures.
- Chromatograms of TCDD mixture on both SP-2330 and Silar-10C columns.
- Summary letter of health efforts of 2,3,7,8-TCDD.
- Form to be signed and returned to Monsanto Research Corporation stating that all safety information was read and understood.
- 1 vial of mixed isomer PE sample for four analyses (actually contained enough sample for seven to eight analyses in case of operator error).
- 1 vial of mixed isomer sample to be used to establish chromatographic conditions if so desired.
- 1 vial containing a specified quantity of 2,3,7,8-TCDD to be used to generate a calibration curve.
- 1 vial containing 2,3,7,8-TCDD fully labeled with carbon-13 to be used to determine recovery correction.
- 1 paint can to store neutralized wastes from the method study.
- 1 fiber-pak to contain disposable gloves, garments, etc., which have the possibility of low-level dioxin contamination.

Appendix B contains copies of the chromatograms and instructions given to the laboratories. The laboratories were requested to complete a report form and send a representative total ion chromatograph of the mixed isomer dioxin PE sample along with the monitored ion chromatogram. The laboratories were informed that the PE sample contained 2,3,7,8-TCDD which needed to be resolved from the 1,2,3,4-TCDD and the 1,4,7,8-TCDD isomers.

PERFORMANCE EVALUATION RESULTS

Fourteen of the 20 laboratories submitted numerical results from the PE samples including chromatograms. Two of the other six

laboratories were able to achieve separation of the isomers as demonstrated by chromatograms but were unable to analyze the PE samples because of sensitivity problems. Four laboratories dropped out of the study because of problems with sensitivity, the glass capillary interface, scheduling, or personnel. Of the 16 laboratories that supplied chromatographic results, seven used a polar SP-2330 (Silar-10C equivalent) column; the other laboratories used less polar DB-5, Carbowax 20M, SE-30, or SE-54 fused silica columns. One of these laboratories switched to Carbowax 20M after encountering difficulties with SP-2330. One of the 14 laboratories did not use Method 613 for the analysis. The laboratories using SP-2330 columns achieved good chromatographic separation of the 2,3,7,8-TCDD from the other isomers, as shown in Figure 1.

The analytical results for the PE samples from the 13 laboratories using Method 613 showed an average 2,3,7,8-TCDD value of 89 parts per trillion ± 38 parts per trillion with an average recovery of 70% ± 27%. The PE sample was analyzed four times by each laboratory. The average intralaboratory variability was ± 12 parts per trillion (average standard deviation). The average intralaboratory variability for recovery was ± 19%.

The total ion chromatograms were examined for each of the laboratories, and the 13 laboratories that successfully completed the Performance Evaluation Study were sent method study samples.

METHOD 613 INTERLABORATORY VALIDATION STUDY

Three target concentration levels of 2,3,7,8-TCDD were chosen for the study: 20 parts per trillion, 80 parts per trillion, and 200 parts per trillion. The low-level target value was selected to be about 10 times the distilled water method detection level.

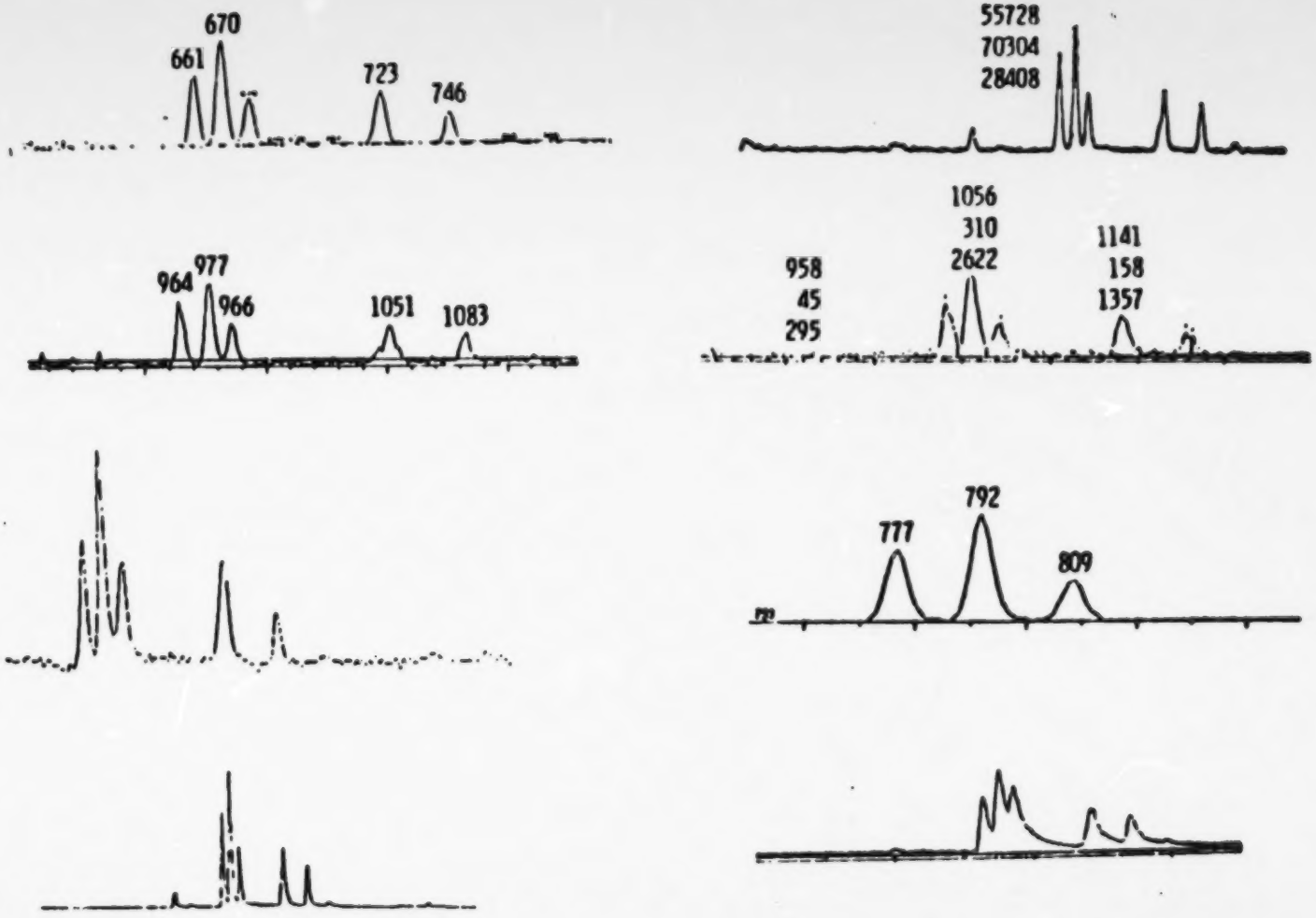

Figure 1. Chromatograms of the performance evaluation mix using SP-2330 columns from eight laboratories.

Note that the original Method 613 was found to have a method detection limit (MDL) of 6 parts per trillion, while the revised Method 613 was found to have an MDL of 1.5 parts per trillion. Both MDL's were determined on a low-resolution quadrupole GC/MS system because most participating laboratories had intended to use this type of instrument.

The target values for the concentrations of the Youden pairs were 20 parts per trillion, 80 parts per trillion, and 200 parts per trillion. The lowest value represents approximately ten times the method detection limit, while the highest value approaches the solubility of TCDD in water. In the method study, the Youden pair concentrations were selected at about ± 5% of the target values, yielding a total of six concentrations. Table 2 lists the true values of the water after spiking and the triplicate analyses data obtained by Monsanto Research Corporation. These analyses were performed by direct injection GC-MS without the sample going through the method extraction and cleanup steps. These analyses were performed prior to sending the ampuls to the participating laboratories. As can be seen in Table 2, the concentration range of this study was 21 ng/L to 202 ng/L.

TABLE 2. TRUE VALUES OF 2,3,7,8-TCDD CONCENTRATIONS
(parts per trillion)

Ampul	True value, ppt	MRC analytical data	
		Mean	Standard deviation
1	202	196.9	10.7
2	182	184.0	8.5
3	81	89.4	3.4
4	73	79.0	5.3
5	26	26.7	3.6
6	21	21.6	1.8

Instructions sent to the participating laboratories included the following information:

General - Method 613 is written as a 2,3,7,8-TCDD specific method. Only the columns that have been demonstrated to separate 2,3,7,8-TCDD from the other 21 tetrachloro-isomers will be allowed. These presently include Silar-10C and SP-2330 columns. SP-2340 has essentially the same chromatographic constants as Silar-10C and will be allowed as an equivalent column.

Samples and water blanks must go through the entire extraction, acid and base washes, alumina column cleanup, and analysis procedure.

Waste Disposal - After combining the acid washes and the base washes and neutralizing the resulting mixture, a precipitate and a clear fluid result. The neutralized liquid fraction is run through the activated charcoal provided by Monsanto Research Corporation, and is subsequently disposed of by sewering. At the end of the study the precipitate and the used charcoal are to be placed in the paint can, heated to dryness, and returned to Monsanto Research Corporation's Dayton laboratory for disposal.

Specific Instructions for Preparation of Samples -

1. Add 1.5 mL of acetone to each sample spiking vial, recap and allow to stand overnight.

2. Add the entire contents of each spiking vial into the bottle containing the water to be spiked. Rinse out the vial with another 2 mL of acetone to assure that all of the spike is added to the water sample.

3. The vial labeled "613 Standard" contains 20.2 µg of unlabeled 2,3,7,8-TCDD. The contents of this vial can be added to 50 mL of tetradecane or other appropriate solvent to produce an analytical standard of 404 ng/mL and diluted to other concentrations as necessary.

4. The ^{13}C TCDD vial contains 2 µg of the labeled compound. The appropriate internal standards should be prepared and used as per Method 613.

5. The samples are to be extracted, concentrated, washed with acid and base, put through the alumina column cleanup and analyzed by GC/MS per Method 613 using a Silar-10C, SP-2330, or SP-2340 capillary column.

Other Instructions -

A Method 613 laboratory questionnaire and a reporting form were sent to the participating laboratories; and were to be returned to Monsanto Research Corporation.

According to the instructions, the six concentrations of 2,3,7,8-TCDD were then spiked into distilled water, tap water, and a surface, lake or pond water all acquired by each of the participants, and into the three different wastewaters supplied to the participants. The wastewaters were selected so that they would be representative of sample matrices in which dioxins might be found. One of the wastewaters was particularly challenging because it readily formed emulsions (Wastewater 1). The study consisted of six concentrations of 2,3,7,8-TCDD to be analyzed in six different water types (totalling 36 analyses) plus the blanks for each water, which represented a total of 42 analyses. The 42 vials (including blank vials) were sent to the participants along with a vial of carbon-13-labeled 2,3,7,8-TCDD, a vial of unlabeled 2,3,7,8-TCDD, and the one-liter bottles of wastewater.

Newly available 60-meter fused silica SP-2330 and SP-2340 columns from Supelco were evaluated and found to give similar separation of the 2,3,7,8-TCDD from the other 21 isomers when compared to the 60-meter glass SP-2330 (as shown in Figures 8 through 10, Appendix B). As a result of this, laboratories that desired to use these

new columns for the method study were given approval to do so. The Supelco Reporter also presented these results to a wider audience [3].

Method Study Results

Of the 13 laboratories that were sent samples for the Method 613 inter-laboratory study, 11 laboratories reported results. A list of the 11 participating laboratories is presented in Table 3. The raw data from the 11 laboratories are presented in Appendix C. Values marked with an asterisk in Appendix C were determined to be outliers using statistical methods described in Section 5 of this report. Three of these laboratories (3, 6 and 11) experienced problems of insufficient sensitivity that precluded low-parts per trillion analyses of the 2,3,7,8-TCDD isomer. A preliminary report on these data was presented earlier [4].

TABLE 3. PARTICIPATING LABORATORIES

Acurex Corporation
Battelle Columbus Laboratories
EAL Corporation
Environmental Research Group
Gulf South Research Institute
IT Enviroscience
Jacobs Laboratories
Midwest Research Institute
PEDCo Environmental
Rocky Mountain Analytical Labs
Southern Research Institute

SECTION 5

STATISTICAL TREATMENT OF DATA

The data obtained from the interlaboratory method validation study were subjected to statistical analyses by Battelle Columbus Laboratories, Columbus, Ohio under EPA contract No. 68-03-2624. The analyses were performed employing USEPA's system of computer programs known as the Interlaboratory Method Validation Study (IMVS) system [5] which was designed to implement the concepts recommended in ASTM Standard D 2777, "Standard Practice for Determination of Precision and Bias of Methods of Committee D-19 on Water" [6]. The analyses conducted using the IMVS system included tests for the rejection of outliers (both whole laboratories for a water-type and individual data points), estimation of mean recovery (accuracy), estimation of single-analyst and overall precision, and tests for the effects of water type on accuracy and precision.

REJECTION OF OUTLIERS

An outlying observation or "outlier" is a data point that appears to deviate markedly from other members of the sample in which it occurs. Outlying data points are very commonly encountered during interlaboratory test programs. If they are not removed, they can result in a distortion of the accuracy and precision statistics that characterize the analytical method. These outlying points cannot be removed indiscriminantly, however, because they may represent an extreme manifestation of the random variability inherent in the method.

ASTM Standard E 178-80, "Standard Practice for Dealing with Outlying Observations" [7] and ASTM Standard D 2777-77 [6] present explicit statistical rules and methods for the identification of outliers.

Data from outlying laboratories for a particular water-type were rejected employing Youden's laboratory ranking test procedure [6, 8] at the 5% level of significance. The data remaining after the laboratory ranking procedure were subjected to individual outlier tests. After zero, missing, "less than," and "nondetect" data were rejected as outliers, the average and standard deviations for the remaining data were calculated. The remaining data were examined for additional outliers employing the outlier rejection test constructed by Thompson [9]. Data rejected as outliers for this study are identified by an asterisk in the table of raw data (Appendix C).

Youden's Laboratory Ranking Procedure

Using the data for each water type, Youden's laboratory ranking test [6, 8] was performed at the 5% level of significance. The Youden laboratory ranking procedure requires a complete set of data from each laboratory within each water type. Missing data from laboratory "i" for water type "j" were replaced by the following procedure. Letting X_{ijk} denote the reported measurement from laboratory "i" for water type "j" and ampul "k," it is assumed that

$$X_{ijk} = \beta_j \cdot C_k^{\gamma_j} \cdot L_i \cdot \varepsilon_{ijk}. \qquad (1)$$

where $i = 1,2,\ldots, n$
$j = 1,2,\ldots, 6$
$k = 1,2,\ldots, 6$

The model components β_j and γ_j are fixed parameters, C_k is the true concentration level associated with ampul "k," the model component L_i is a random factor which accounts for the systematic error associated with laboratory "i," and the model component ε_{ijk} is the random factor which accounts for the within laboratory error.

Taking natural logarithms, it follows that

$$\ell n\ X_{ijk} = \ell n\ \beta_j + \gamma_j\ \ell n\ C_k + \ell n\ L_i + \ell n\ \varepsilon_{ijk} \qquad (2)$$

which is a linear regression model with dependent variable $\ell n\ X_{ijk}$ and independent variable $\ell n\ C_k$. (Details and justification for this model are discussed in the later section of this report entitled "Comparison of Accuracy and Precision Across Water Types.")

The natural logarithms of the individual laboratory's data were regressed against the natural logarithms of the true concentration levels for the six ampuls in each water type. The predicted values $\ell n\ X_{ijk}$ were obtained from the regression equation, and the missing values for X_{ijk} were estimated by $X_{ijk} = \exp(\ell n\ X_{ijk})$. (For complete details of this procedure, see Reference 5.)

An example of the use of Youden's laboratory ranking procedure is presented in Table 4, where the rankings of the values for 2,3,7,8-TCDD in water 4 are listed for each laboratory and for ampuls 1 through 6. For 11 laboratories and six ampuls, the upper and lower critical limits of the sums of the rankings are 57 and 15. If the sum of the rankings of any laboratory equals or exceeds 57 or is equal or less than 15, that laboratory's data are rejected for all determinations for that analyte (2,3,7,8-TCDD) in that water (water 4). Therefore from Table 4 it is apparent that the water 4 data from Laboratory 3 must be rejected. After the laboratory ranking test had been conducted, the estimated missing data were then removed from the data sets.

TABLE 4. YOUDEN LABORATORY RANKING PROCEDURE FOR 2,3,7,8-TCDD DATA IN WATER 4

Laboratory No.	Ranking value						Cumulative score
	Ampul 1	Ampul 2	Ampul 3	Ampul 4	Ampul 5	Ampul 6	
1	8	4	3	6	5	4	30
2	6	3	5	4	3	6.5	27.5
3	11	10	10	10	9.5	10	60.5[a]
4	3	2	1	1	2	8	17
5	10	9	8	2	4	5	38
6	2	11	10	10	9.5	10	52.5
7	4	5	2	3	1	1	16
8	5	1	10	10	9.5	10	45.5
9	7	6	4	5	6	3	31
10	9	7	6	7	7	6.5	42.5
11	1	8	7	8	9.5	2	35.5

[a]Laboratory rejected because upper critical limit of cumulative score exceeds 57.

Test for Individual Outliers

The data remaining after rejection of zero, missing, "less than" and "nondetect" data were subjected to an individual outlier test based on calculation of the average value, $\bar{X}$, for each ampul and the standard deviation of the remaining values.

The criterion for rejection of individual outliers is based upon calculation of Thompson's T-value [6, 9]. In these calculations the mean recovery, $\bar{x}$, is given by

$$\bar{x} = \frac{1}{n} \sum_{i=1}^{n} x_i \qquad (3)$$

and the standard deviations, "s," is given by

$$s = \sqrt{\frac{1}{n-1} \sum_{i=1}^{n} (x_i - \bar{x})^2} \qquad (4)$$

where x_i = individual analyses
n = number of retained analyses values in the ampul set

Thompson's T is defined by

$$T = \frac{x_e - \bar{x}}{S} \qquad (5)$$

where x_e is the retained x_i value farthest away from the mean ($\bar{x}$) of the set of retained values.

The data point may be rejected if the value of T calculated exceeds the critical value for T (two-sided test, 5% significance level) as presented in Table 5. If the extreme value is rejected as an outlier, the test is repeated for the next extreme value among the remaining data until the value being tested is less than the critical T-value.

As shown in Table 6, four additional data points are identified as outliers by the Thompson's test for the illustrative example of the analyte 2,3,7,8-TCDD in water 4. In summary, of the original 66 data points for 2,3,7,8-TCDD in water 4 (11 laboratories x six ampuls), all data points for laboratory 3 were rejected on the basis of Youden's laboratory ranking procedure (total of six points), and four additional data points were found to be outliers based on the T-test (for a total of the 10 data points that are identified by an asterisk as outliers in Appendix C).

TABLE 5. CRITICAL VALUES FOR THOMPSON'S T (TWO-SIDED TEST) WHEN STANDARD DEVIATION IS CALCULATED FROM THE SAME SAMPLES

Number of observations, n	5% significance level
3	1.15
4	1.48
5	1.71
6	1.89
7	2.02
8	2.13
9	2.21
10	2.29
11	2.36
12	2.41
13	2.46
14	2.51
15	2.55
16	2.58
17	2.62
18	2.65
19	2.68
20	2.71

TABLE 6. RESULTS OF TEST FOR INDIVIDUAL OUTLIERS (2,3,7,8-TCDD IN WATER 4)

Ampul	Lab	Extreme value (x_e)	Mean ($\bar{x}$)	Standard deviation (s)	Calculated T $\frac{x_e - \bar{x}}{s} = T$	Number of points (n)	Critical T (T_c)	Decision
6	4[a]	11.00	17.71	3.04	2.21	7	2.02	Reject
6	7	33.00	19.62	6.08	2.20	8	2.13	Reject
3	4	186.00	83.62	41.94	2.44	8	2.13	Reject
4	4	182.00	77.02	44.10	2.38	8	2.13	Reject

[a]Laboratory 4, ampul 6 value rejected after rejection of Laboratory 7, ampul 6 value.

STATISTICAL SUMMARIES

After the outlier rejection tests were performed, several summary statistics were calculated employing the remaining data for each ampul (single analyte, single concentration, single water matrix). These statistics include:

- number of retained data points, n
- mean recovery of the retained data, $\bar{x}$
- accuracy as a percent of relative error, % RE
- overall absolute standard deviation, S
- percent relative overall standard deviation, % RSD
- absolute single-analyst standard deviation, SR
- percent relative single-analyst standard deviation, % RSD-SA

All of these statistics, except the single-analyst absolute and relative standard deviations, were calculated using the retained data for each ampul. The basic statistical formulas used for these calculations are given below where X_1, X_2,..., X_n denote the values for the n retained data points for a given ampul.

Mean Recovery ($\bar{X}$):

$$\bar{X} = \frac{1}{n} \sum_{i=1}^{n} X_i \qquad (3)$$

Accuracy as a % Relative Error:

$$\% \text{ RE} = \frac{\bar{X} - \text{true value}}{\text{true value}} \times 100 \qquad (6)$$

Overall Standard Deviation:

$$S = \sqrt{\frac{1}{n-1} \sum_{i=1}^{n} (X_i - \bar{X})^2} \qquad (4)$$

and

Percent Relative Overall Standard Deviation:

$$\%RSD = \frac{S}{\bar{X}} \times 100 \qquad (7)$$

The overall standard deviation, S, indicates the precision associated with measurements generated by a group of laboratories. This represents the broad variation in the data collected in a collaborative study. A measure of how well an individual analyst can expect to perform in his own laboratory is another important measure of precision. This single-analyst precision, denoted by SR, is measured by

$$SR = \sqrt{\frac{1}{2(m-1)} \sum_{i=1}^{m} (D_i - \bar{D})^2} \qquad (8)$$

where m = number of retained Youden-paired observations

D_i = difference between the observations in the i^{th} pair

$\bar{D}$ = the average of the D_i values

The Youden-pair design employed in this study permits the calculation of single-analyst precision without duplicate measurements on the same sample and helps to avoid the well-intentioned manipulation of data that can occur when laboratories make duplicate analyses.

The percent relative standard deviation for the single-analyst precision is calculated by

$$\%RSD\text{-}SA = \frac{SR}{\bar{X}^*} \times 100 \qquad (9)$$

where $\bar{X}^*$ is the average of the two mean recoveries corresponding to the two ampuls defining the particular Youden pair. These summary statistics are presented in Table 7 for 2,3,7,8-TCDD in the six water matrices.

REGRESSION ANALYSIS OF BASIC STATISTICS

Frequently, systematic relationships exist between the mean recovery statistics and the true concentration levels across ampuls, and between the precision statistics and the mean recovery statistics. Given a plot of precision values versus concentration levels, a smooth curve drawn through the points can show that the precision (1) is constant and does not vary with level; (2) varies directly with level in a linear manner; or (3) varies with level in a curvilinear fashion.

In order to derive statements for method accuracy and method precision, the basic statistics were regressed assuming linear relationships, fitting the data to a line using weighted least squares. The weights were chosen to be (1) inversely related to the true concentration in the case of accuracy; and (2) inversely related to the mean recovery in the case of precision. The inverse weightings were employed to moderate the influence of the high Youden-pair data. The results of the regression analyses are discussed below.

TABLE 7. STATISTICAL SUMMARY FOR 2,3,7,8-TCDD ANALYSES BY WATER TYPE

	WATER 1		WATER 2		WATER 3		WATER 4		WATER 5		WATER 6	
LOW YOUDEN PAIR	5	6	5	6	5	6	5	6	5	6	5	6
NUMBER OF DATA POINTS	8	8	4	6	6	6	7	6	8	7	8	9
TRUE CONC (C) NG/L	26.00	21.00	26.00	21.00	26.00	21.00	26.00	21.00	26.00	21.00	26.00	21.00
MEAN RECOVERY (X)	23.19	20.46	25.43	22.07	25.51	20.51	29.59	18.83	23.36	23.14	22.33	21.94
ACCURACY(%REL ERROR)	-10.80	-2.56	-2.17	5.08	-1.87	-2.32	13.80	-10.34	-10.15	10.18	-14.10	4.48
OVERALL STD DEV (S)	4.06	3.96	4.66	6.05	4.30	2.70	4.32	0.77	5.61	8.74	3.67	6.02
OVERALL REL STD DEV, %	17.50	19.34	18.30	27.41	16.84	13.15	14.59	4.10	24.00	37.76	16.45	27.46
SINGLE STD DEV, (SR)	4.27		8.28		4.38		1.80		4.22		4.23	
ANALYST REL DEV, %	19.58		34.85		19.03		7.44		18.16		19.09	
MEDIUM YOUDEN PAIR	3	4	3	4	3	4	3	4	3	4	3	4
NUMBER OF DATA POINTS	7	8	6	7	6	7	7	7	8	8	8	8
TRUE CONC (C) NG/L	81.00	73.00	81.00	73.00	81.00	73.00	81.00	73.00	81.00	73.00	81.00	73.00
MEAN RECOVERY (X)	64.40	61.98	67.00	66.46	64.35	60.42	68.99	62.02	70.16	60.38	66.22	60.56
ACCURACY(%REL ERROR)	-20.49	-15.09	-17.28	-8.95	-20.55	-17.23	-14.83	-15.04	-13.38	-17.28	-18.24	-17.05
OVERALL STD DEV (S)	16.49	14.33	14.39	25.28	15.30	13.31	7.36	13.04	10.95	6.76	5.66	8.94
OVERALL REL STD DEV, %	25.60	23.11	21.48	38.03	23.78	22.02	10.67	21.02	15.60	11.20	8.54	14.76
SINGLE STD DEV, (SR)	8.70		5.55		7.85		9.20		5.78		9.73	
ANALYST REL DEV, %	13.77		8.32		12.58		14.05		8.85		15.35	
HIGH YOUDEN PAIR	1	2	1	2	1	2	1	2	1	2	1	2
NUMBER OF DATA POINTS	7	7	6	6	6	6	10	9	8	8	9	7
TRUE CONC (C) NG/L	202.00	182.00	202.00	182.00	202.00	182.00	202.00	182.00	202.00	182.00	202.00	182.00
MEAN RECOVERY (X)	169.73	183.53	162.76	158.78	167.19	158.71	210.66	176.30	169.62	165.29	176.37	136.69
ACCURACY(%REL ERROR)	-15.97	0.84	-19.43	-12.76	-17.23	-12.79	4.29	-3.13	-16.03	-9.18	-12.69	-24.90
OVERALL STD DEV (S)	28.55	23.38	27.60	27.37	37.86	17.69	58.10	54.88	30.94	44.91	52.73	31.00
OVERALL REL STD DEV, %	16.82	12.74	16.96	17.24	22.64	11.15	27.58	31.13	18.24	27.17	29.90	22.68
SINGLE STD DEV, (SR)	26.78		14.70		17.46		48.48		14.77		28.43	
ANALYST REL DEV, %	15.16		9.14		10.71		25.06		8.82		18.16	

WATER LEGEND

1 - DISTILLED WATER
2 - TAP WATER
3 - SURFACE WATER
4 - WASTE WATER 1
5 - WASTE WATER 2
6 - WASTE WATER 3

Statements of Method Accuracy

The accuracy of Method 613 is characterized by comparing the mean recovery of the analyte, $\bar{X}$, to the true concentration level of the compound, C, in the ampul. The IMVS program conducts these calculations via matrix algebra, where weighted least-squares linear regression of $\bar{X}$ versus C is conducted with weights chosen to be inversely related to the square of the true concentration levels (see Reference 5, page 108 for details). This method is equivalent to that suggested by Britton [10], where the linear regression for $\bar{X}$ versus C is achieved by using the customary least-squares algorithm to fit:

$$\frac{\bar{X}}{C} = a + b\left(\frac{1}{C}\right) \qquad (10)$$

which can then be converted to the desired relationships by multiplying through by C, giving:

$$\bar{X} = aC + b \qquad (11)$$

These equations were presented earlier in Table 1.

If the intercept "b" associated with the fitted line is negligible (i.e., essentially zero), then the slope "a" provides a unique value that represents the percent recovery over all of the concentration levels.

Statements of Method Precision

The precision of Method 613 is characterized by comparing the overall and single-analyst standard deviations to the mean recovery, $\bar{X}$. The IMVS program conducts these calculations via matrix algebra,

where a weighted least-squares linear regression of S and SR versus $\bar{X}$ is conducted with weights chosen to be inversely proportional to the square of the mean recovery (see Reference 5, page 108 for details). This method is equivalent to that suggested by Britton [10], where the linear regressions for S and SR versus C are achieved by using the customary least-squares procedure to fit the equations,

$$\frac{S}{C} = c + d\left(\frac{1}{C}\right) \tag{12}$$

In this study, however, the regression was conducted versus $\bar{X}$ as follows:

$$\frac{S}{\bar{X}} = c + d\left(\frac{1}{\bar{X}}\right) \tag{13}$$

which is then converted by multiplying through by $\bar{X}$ to yield the linear relationships

$$S = a\bar{X} + b \tag{14}$$

and

$$SR = c\bar{X} + d \tag{15}$$

These equations are also presented in Table 1.

If the intercepts "b" and "d" are negligible, then the slopes "a" and "c" are good approximations to the overall and single-analyst percent relative standard deviations, respectively. These, in turn, are measures of the method precision.

As a test of the relative magnitudes of the intercept values, average values of S, SR, and $\bar{X}$ were calculated from the data in Table 7 over the three concentration levels reported in this study. The average values of the statistical quantities and the percentage of these average values represented by the intercepts of the linear regression equations are presented in Table 8. The intercepts represent a substantial portion of the average values for the cases of overall standard deviation for analyses in wastewaters 1 and 2, and for the cases of single-analyst standard deviation for analyses in distilled water, tap water, and wastewaters 1 and 2. In these cases, the slopes of the linear regression equations may not give representative values for precision of the 2,3,7,8-TCDD analyses, because the intercepts cannot be considered negligible.

COMPARISON OF ACCURACY AND PRECISION ACROSS WATER TYPES

It is possible that the accuracy and precision of Method 613 depend on the water type analyzed. The summary statistcs $\bar{X}$, S, and SR are calculated separately for each concentration level within each water type. They can be compared across water types to obtain information about the effects of water type on accuracy and precision. However, the use of these summary statistics in this manner has several disadvantages. First, it is cumbersome because there are 36 mean recovery statistics ($\bar{X}$) (six ampuls x six waters), 36 precision statistics (S), and 18 precision statistics (SR) calculated for 2,3,7,8-TCDD. Comparison of these statistics across concentration levels and across water types becomes unwieldy. Second, the statistical properties of this type of comparison procedure are difficult to determine. Finally, due to variation associated with $\bar{X}$, S, and SR, comparisons based on these statistics can lead to inconsistent conclusions about the effect of water type. For example, distilled water may produce a significantly lower value than drinking

TABLE 8. MAGNITUDES OF THE INTERCEPTS OF THE LINEAR REGRESSION EQUATIONS

Water matrix	Statistical quantities	Average value over all concentrations	Percentage of average value represented by intercept
Distilled water	Mean recovery, $\bar{X}$	87.25	1.7
	Overall standard deviation, S	15.13	1.9
	Single-analyst standard deviation, SR	13.25	9.7
Tap water	Mean recovery, $\bar{X}$	83.74	5.8
	Overall standard deviation, S	17.56	4.6
	Single-analyst standard deviation, SR	9.51	81.8
Surface water	Mean recovery, $\bar{X}$	87.49	3.9
	Overall standard deviation, S	17.82	6.2
	Single-analyst standard deviation, SR	13.40	16.8
Wastewater 1	Mean recovery, $\bar{X}$	94.40	1.1
	Overall standard deviation, S	23.08	20.1
	Single-analyst standard deviation, SR	19.80	22.3
Wastewater 2	Mean recovery, $\bar{X}$	84.26	5.1
	Overall standard deviation, S	17.82	17.5
	Single-analyst standard deviation, SR	9.53	28.4
Wastewater 3	Mean recovery, $\bar{X}$	80.65	5.3
	Overall standard deviation, S	18.01	0.1
	Single-analyst standard deviation, SR	14.13	3.5

water for the precision statistic S at a high concentration, but a significantly higher value for S at a low concentration.

An alternative approach, described in detail in Reference 5, has been developed to test for the effects of water type. This alternative approach is based on the concept of summarizing the average effect of water type across concentration levels rather than studying the local effects at each concentration level. If significant differences are established by this alternative technique, then the summary statistics can be used for further local analysis.

The test for the effect of water type is based on the following statistical model. If X_{ijk} denotes the measurement reported by laboratory "i," for water type "j," and ampul "k," then

$$X_{ijk} = \beta_j \cdot C_k^{\gamma_j} \cdot L_i \cdot \varepsilon_{ijk} \qquad (1)$$

where $i = 1,2,\ldots, n$
$j = 1,2$
$k = 1,2,\ldots, 6$

The model components β_j and γ_j are fixed parameters that determine the effect of water type j on the behavior of the observed measurements (X_{ijk}). The parameter C_k is the true concentration level associated with ampul "k." The model component L_i is a random factor that accounts for the systematic error associated with laboratory "i." The model component ε_{ijk} is the random factor which accounts for the within-laboratory error.

The model is designed to approximate the global behavior of the data. The multiplicative structure was chosen because of two important properties. First, it allows for a possible curvilinear relationship between the data (X_{ijk}) and the true concentration level (C_k) through the use of the exponent γ_j on C_k. This makes the model more flexible in the data and the concentration level

C_k in this model. This property is important because it is typical of interlaboratory data collected under conditions where the true concentration levels vary widely.

Accuracy is related directly to the mean recovery or expected value of the measurements (X_{ijk}). The expected value for the data modeled by Equation (1) is

$$E(X_{ijk}) = \beta_j \cdot C_k^{\gamma_j} \cdot E(L_i \cdot \varepsilon_{ijk}) \qquad (16)$$

Precision is related to the variability in the measurements (X_{ijk}). The variance of the data modeled by Equation (1) is

$$Var(X_{ijk}) = \left[\beta_j \cdot C_k^{\gamma_j}\right]^2 Var(L_i \cdot \varepsilon_{ijk}), \qquad (17)$$

which is an increasing function of C_k. (See Reference 5 for a complete discussion of this model.)

The accuracy and precision of Method 613 depend upon water type through Equations 16 and 17 and the parameters β_j and γ_j. If β_j and γ_j vary with j (i.e., vary across water type), then the accuracy and precision of the method also vary across water type.

To determine if these parameters vary across water type and to compare their values, they must be estimated from the laboratory data using regression techniques. Equation 1 represents the basic model. However, taking natural logarithms of both sides of Equation 1, the following straight line regression model is obtained:

$$\ell n\, X_{ijk} = \ell n\, \beta_j + \gamma_j\, \ell n\, C_k + \ell n\, L_i + \ell n\, \varepsilon_{ijk} \qquad (2)$$

The parameter $\ell n\, \beta_j$ is the intercept and γ_j the slope of the regression line associated with water type j. It is assumed that

$\ell n\ L_i$ is normally distributed with mean 0 and variance σ_L^2, that $\ell n\ \varepsilon_{ijk}$ is normally distributed with mean 0 and variance σ_ε^2, and that $\ell n\ L_i$ and $\ell n\ \varepsilon_{ijk}$ are independent terms.

Based on Equation 2, the comparison of water types reduces to the comparison of straight lines. Distilled water is viewed as a control, and each of the remaining lines is compared directly to the line for distilled water.

Using the data on the log-log scale and regression techniques, the parameter $\ell n\ \beta_j$ (hence β_j) and γ_j can be estimated. The estimates are then used to formally test the null versus alternative hypotheses

$$H_0: \quad \ell n\ \beta_j - \ell n\ \beta_1 = 0 \text{ and } \gamma_j - \gamma_1 = 0 \text{ for } j = 2 \tag{18}$$

versus

$$H_A: \quad \ell n\ \beta_j - \ell n\ \beta_1 \neq 0 \text{ and/or } \gamma_j - \gamma_1 \neq 0 \text{ for some } j = 2 \tag{19}$$

The null hypothesis (H_0) is tested against the alternative hypothesis (H_A) using an F-statistic. The probability of obtaining the value of an F-statistic as large as the value that was actually observed, Prob(F > F OBS), is calculated under the assumption that H_0 is true. H_0 is rejected in favor of H_A if Prob(F > F OBS) is less than 0.05.

If H_0 is not rejected, there is no evidence in the data that the β_j vary with j or that the γ_j vary with j. Therefore, there is no evidence of an effect due to water type on the accuracy or precision of the method. If H_0 is rejected, some linear combination of the differences ($\ell n\ \beta_j - \ell n\ \beta$) and ($\gamma_j - \gamma_1$) is statistically different from zero. However this does not guarantee that will be a statistically significant direct effect attributable to any

specific water type since the overall F test can be overly sensitive to minor systematic effects common to several water types. The effect due to water type is judged to be statistically significant only if one of the differences ($\ell n\ \beta_j - \ell n\ \beta_1$) and/or ($\gamma_j - \gamma_1$) is statistically different from zero. This is determined by checking the simultaneous 95% confidence intervals which are constructed for each of these differences. Each true difference can be stated to lie within its respective confidence interval with 95% confidence. If zero is contained within the confidence interval, there is no evidence that the corresponding difference is significantly different from zero.

If at least one of the confidence intervals for the differences ($\ell n\ \beta_j - \ell n\ \beta_1$) or ($\gamma_j - \gamma_1$) fails to include zero, the statistical significance of the effect due to water type has been established. However, establishment of a statistically significant effect due to water type does not necessarily mean that the effect is of practical importance, which is related to the size and interpretation of the differences.

The interpretation of the differences involves comparing the mean recovery and standard deviation for each water type to the mean recovery and standard deviation obtained for distilled water. These comparisons are made on a relative basis. The mean recovery for water type j is given by Equation 16. The mean recovery for water type j is compared to that for distilled water (j = 1) on a relative basis by

$$\frac{E(X_{ijk})}{E(X_{ilk})} = \frac{\beta_j\ C_k^{\gamma_j}\ E(L_i \cdot \varepsilon_{ijk})}{\beta_1\ C_k^{\gamma_1}\ E(L_i \cdot \varepsilon_{ilk})} = \frac{\beta_j}{\beta_1} C_k^{(\gamma_j - \gamma_1)} \qquad (20)$$

(The ratio of the standard deviations would be equivalent to Equation 20. Therefore, the interpretation of the effect on precision is the same as that for the effect on mean recovery).

The ratio in Equation 20 is a measure of the relative difference in mean recovery between water type j and distilled water. It is composed of two parts: (a) β_j/β_1, which is independent of the true concentration level (i.e., the constant bias), and (b) $C_k^{(\gamma_j - \gamma_1)}$ which depends upon the true concentration level (i.e., the concentration dependent bias). If $(\gamma_j - \gamma_1)$ is zero, the relative difference in mean recovery is just β_j/β_1, which is independent of concentration level (C_k). It can then be stated that the mean recovery of water type j is (β_j/β_1) x 100 percent of the mean recovery for distilled water. If $(\gamma_j - \gamma_1)$ is not zero, the mean recovery of water type j is $([\beta_j/\beta_1] \cdot C_k^{[\gamma_j - \gamma_1]})$ x 100 percent of that for distilled water, and therefore it depends on the true concentration level (C_k).

In order to illustrate these points, consider the following example. Suppose that a significant F-value has been obtained, and the confidence intervals for all the differences contain zero except for water type 5. For water type 5, the point estimate for $(\ell n\ \beta_5 - \ell n\ \beta_1)$ is -0.38, and the confidence interval for $(\ell n\ \beta_5 - \ell n\ \beta_1)$ is (-0.61, -0.07). The point estimate for $(\gamma_5 - \gamma_1)$ is -0.02, and the confidence interval for $(\gamma_5 - \gamma_1)$ is (-0.03, 0.18). In this case, a statistically significant effect due to water type has been established which involves only water type 5. The practical significance of this effect is judged by considering Equation 20. The ratio of mean recoveries for water type 5 and distilled water is given by

$$\frac{E(X_{i5k})}{E(K_{i1k})} = \frac{\beta_5}{\beta_1} C_k^{(\gamma_5 - \gamma_1)} \qquad (21)$$

and the ratio of the standard deviations is given by

$$\sqrt{\frac{Var(X_{ijk})}{Var(X_{i1k})}} = \frac{\beta_5}{\beta_1} C_k^{(\gamma_5 - \gamma_1)} \qquad (22)$$

Because the confidence interval for ($\gamma_5 - \gamma_1$) contains zero, this difference is assumed to be insignificant and is set to zero. Therefore, Equations 21 and 22 reduce to β_5/β_1. The point estimate for ($\ell n\ \beta_5 - \ell n\ \beta_1$) was -0.38. Therefore, the point estimate for β_5/β_1 is 0.68, and the mean recovery for water type 5 is estimated to be 68% of the mean recovery for distilled water. Similarly, the standard deviation for the data for water type 5 is estimated to be 68% of the standard deviation for distilled water. Since the 95% confidence interval for ($\ell n\ \beta_5 - \ell n\ \beta_1$) was (-0.61, -0.07), any value in the interval (0.54, 0.93) is a reasonable estimate for β_5/β_1, and the mean recovery (standard deviation) for water type 5 can be claimed to be from 54% to 93% of the mean recovery (standard deviation) for distilled water. The practical significance of the effect due to water type 5 would depend upon the importance of a mean recovery (standard deviation) which is between 54% and 93% of the mean recovery (standard deviation) observed for distilled water.

The comparison of accuracy and precision across water types just discussed, is based on the assumption that Equation (1) approximately models the data. It is clear that in practical monitoring programs of this type such models cannot model the data completely in every case. This analysis, therefore, is viewed as a screening procedure which identifies those cases where differences in water types are likely to be present. A more detailed, local analysis can then be pursued using the basic summary statistics for precision and accuracy.

The data from analysis of variance procedure is presented in Table 9.

TABLE 9. EFFECT OF WATER TYPE ON 2,3,7,8-TCDD ANALYSES

** POINT ESTIMATES **

DISTILLED WATER SLOPE:GAMMA(1) = 1.00241

WATER	INTERCEPT(WATER-DISTILLED)	SLOPE(WATER-DISTILLED)
2	.4694	-.1020
3	.3588	-.0781
4	.2507	-.0410
5	.3068	-.0651
6	.3291	-.0839

** ANALYSIS OF VARIANCE **

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB
REG(DISTILLED)	1	171.49933	171.49933		
REG(WATER/DISTILLED)	10	.50271	.05027	1.28	.2447
ERROR	238	9.37675	.03940		
TOTAL	249	181.37879			

** TABLE OF 95% CONFIDENCE INTERVALS FOR THE DIFFERENCES BETWEEN INTERCEPTS AND THE DIFFERENCES BETWEEN SLOPES **

WATER	INTERCEPT(WATER-DISTILLED) ESTIMATE	INTERVAL	SLOPE(WATER-DISTILLED) ESTIMATE	INTERVAL
2	.4694	(-.1764 , 1.1152)	-.1020	(-.2503 , .0463)
3	.3588	(-.2687 , .9863)	-.0781	(-.2230 , .0668)
4	.2507	(-.3529 , .8542)	-.0410	(-.1784 , .0964)
5	.3068	(-.2838 , .8974)	-.0651	(-.2012 , .0709)
6	.3291	(-.2464 , .9046)	-.0839	(-.2171 , .0493)

NOTE: IF ZERO IS CONTAINED WITHIN A GIVEN CONFIDENCE INTERVAL THEN THERE IS NO STATISTICAL SIGNIFICANCE BETWEEN DISTILLED WATER AND THE CORRESPONDING WASTE WATER FOR THE ASSOCIATED PARAMETER(INTERCEPT/SLOPE).

THE SLOPE AND INTERCEPT ESTIMATES FROM THIS ANALYSIS ARE NOT THE SAME AS THOSE OBTAINED FROM THE PRECISION AND ACCURACY REGRESSIONS PERFORMED EARLIER.

SECTION 6

DISCUSSION

The objective of this study was to characterize the performance of Method 613 in terms of accuracy, overall precision, single-analyst precision, and the effect of water type on accuracy and precision.

One measure of the performance of the method is that 26% of the 360 analytical values were rejected as outliers. This does not include the data from laboratory 3, all of which were rejected since Method 613 was not followed. Table 10 summarizes the number of data points rejected from each laboratory along with the suspected reasons for the outliers. Six of the laboratories did not encounter major problems with the method, and in these cases less than 2% of the data were rejected as outliers.

TABLE 10. SUMMARY OF REJECTED DATA POINTS

Laboratory number	Number of outliers	Problems
1	1	None
2	1	None
3	36	All data rejected. Did not follow method.
4	31	Alumina cleanup.
5	0	None
6	31	No recovery on C_{12}^{13} internal standard.
7	2	None
8	11	Lack of sensitivity at low levels.
9	0	None
10	0	None
11	18	Alumina cleanup.

RECOVERY (ACCURACY) AND RELATIVE STANDARD DEVIATION (PRECISION)

From the statistical summary and the regression equations presented earlier in Tables 1 and 7, the percent recovery, percent relative standard deviation, and percent relative standard deviation for single-analyst are summaried in Tables 11 through 13. Each table gives the percentages for each Youden-pair and each water type. Table 11 indicates a high percent recovery at the lowest Youden-pair. This is probably due to the slight positive bias as seen by the intercepts in the regression equations in Table 1. The positive bias is expected to be more pronounced at the lower concentrations. Tables 11 through 13 indicate random distribution. There are no patterns in which a single Youden-pair is always the most or least accurate or precise. The accuracy and precision of the method are satisfactory. The problems encountered by the laboratories, as given by their responses to the questionnaire, probably account for the slightly lower than expected recovery and precision.

EFFECTS OF WATER TYPES

The comparison of accuracy and precision across water types is summarized in Table 14, where the observed F values and the probability of exceeding the F values are entered for 2,3,7,8-TCDD in the six water matrices.

The F-test suggests no statistically significant effect due to water type since [P(F > observed F)] >0.05.

SUMMARY OF RESPONSES TO LABORATORY QUESTIONNAIRE

Problems Associated with Extraction/Concentration of Samples

Most laboratories encountered emulsion problems in extracting wastewater 1. The emulsion was usually broken by either

TABLE 11. PERCENT RECOVERIES (ACCURACY) OF METHOD 613

Water matrix	Percent recovery			
	Low Youden pair	Middle Youden pair	High Youden pair	Average over six concentrations
Distilled water	93	82	92	89
Tap water	101	87	84	91
Surface water	105	86	89	93
Wastewater 1	102	85	101	96
Wastewater 2	100	85	86	90
Wastewater 3	95	82	81	86
Average over water matrices	99	85	89	91

TABLE 12. PERCENT RELATIVE OVERALL STANDARD DEVIATION (INTERLABORATORY PRECISION) OF METHOD 613

Water matrix	% RSD			
	Low Youden pair	Middle Youden pair	High Youden pair	Average over six concentrations
Distilled water	18	24	15	19
Tap water	23	30	17	23
Surface water	21	25	18	22
Wastewater 1	9	16	29	18
Wastewater 2	31	13	23	22
Wastewater 3	22	12	26	20
Average over water matrices	21	20	21	21

TABLE 13. PERCENT RELATIVE SINGLE-ANALYST STANDARD DEVIATIONS (INTRALABORATORY PRECISION) OF METHOD 613

Water matrix	% RSD-SA			
	Low Youden pair	Middle Youden pair	High Youden pair	Average over six concentrations
Distilled water	20	14	15	16
Tap water	35	8	9	17
Surface water	16	21	13	17
Wastewater 1	7	14	25	16
Wastewater 2	18	9	11	13
Wastewater 3	19	15	18	18
Average over water matrices	19	14	15	16

TABLE 14. SUMMARY OF TEST FOR DIFFERENCES ACROSS WATER TYPES

Compound	Observed F-value	P[F > observed F]	F-test statistically significant at the 5% level?
2,3,7,8-TCDD	1.28	0.24	No

centrifugation or by agitation with a wire. Laboratory 2 reported a gelatinous precipitate formation with wastewater 2. Laboratory 4 measured the pH of wastewater 2 to be greater than 10 and suggested that the method should specify neutralization procedures to be employed. Laboratory 1 suggested that the methylene chloride extract be dried before the Kuderna-Danish concentration step because residual water reduced the effectiveness of boiling chips used in the concentration step.

Problems Associated with Acid or Base Washes

No laboratories reported problems with acid or base washes. However, one laboratory reported that this step was very slow and required caution due to the handling of the concentrated acid and base solutions.

Problems Associated with Alumina Column Cleanup Procedure

Nine of 11 laboratories reported no problems with the cleanup procedure. One laboratory recommended that the method specify the range of alumina column flow rates acceptable for quantitative recovery. Two laboratories encountered problems in the cleanup stage. Laboratory 4 used an activated alumina from a different supplier for wastewaters B and C and could not elute the 2,3,7,8-TCDD, using the 20:80 methylene chloride hexane solution from those alumina columns. When 100% methylene chloride was employed on two samples, the 2,3,7,8-TCDD was eluted.

Laboratory 11 found that when a portion of one lot of alumina was activated for 16 hours at 140°C, acceptable analytical results were obtained on wastewaters B and C. When the same alumina was activated by heating for 64 hours at 140°C, little or no data were obtained for the other four of the six water types. These results suggest that the activity of the alumina is critical and can give variable results.

Problems Encountered in Analysis of Samples (Interferences, Sensitivity)

Responses to the question on this problem varied widely. Laboratory 1 encountered some interference with the 320 ion on one sample and used the 322 ion instead. The same laboratory experienced interference in the tagged ^{37}Cl isomer with all wastewater 2 samples. Laboratory 2 experienced column loading problems even

though the column and inlet were deactivated. This was especially true at lower levels of 2,3,7,8-TCDD. Laboratory 3 experienced sensitivity problems (could not detect below 45 ppt). Difficulties in seeing the 50 ppt ^{13}C-tagged isomer led to use of D_{10}-anthacene as an internal standard (added just before injection). For this reason, all of the laboratory 3 data were rejected, since they did not follow the prescribed method. Laboratory 5 had problems in reaching the detection limits and assumed that the analytical system was adsorbing the isomer. Laboratory 6 experienced no recovery of ^{13}C-TCDD in many samples. Laboratory 8 experienced either no detection or low recovery on all wastewater A samples. Laboratory 10 reported that signals occasionally approached their detection limits. Laboratories 5 and 7 favored the use of fused silica columns to ease column installation problems and to allow the column to be placed directly in the source block.

Problems Encountered in Testing Waste for Disposal

Laboratories 2 and 7 suggested a coarser grade of carbon in the waste disposal procedure. The activated carbon was too fine for good flow. In one case, crystals formed and plugged the carbon. Laboratory 7 stated that the procedure generated too much salt product. Laboratory 10 complained that the large volumes of H_2SO_4 were difficult to handle, but felt that the carbon adsorbent approach was better than heating to dryness.

Other Problems/Suggestions

Laboratory 1 experienced a cracked weld in the inlet system that resulted in oxidation of the Carbowax deactivation coating. Successful deactivation was achieved by employing 0.1% SP-2330 in methylene chloride solution. Laboratory 4 found that the response factor was linear only above 600 pg and exhibited a standard deviation of less than 10% at any concentration. The peak areas of ^{13}C-TCDD showed a 21% standard deviation and indicated recoveries

between 18% and 89%. Laboratory 7 suggested that Method 613 specify the use of two independent internal standards as a quality assurance measure to detect possible errors in known concentration, evaporated concentration, or incomplete dissolution of standards.

Table 15 lists the laboratories and their instrumentation and conditions.

TABLE 15. GC/MS CONDITIONS EMPLOYED IN THE METHOD 613 STUDY

Laboratory No.	GC/MS instrument	GC column-length, m	Carrier gas	Carrier gas linear flow-rate, cm/s
1	Hewlett-Packard 5985	SP2330-60m	Helium	20 cm/sec
2	Finnigan 3200 (RDS data system)	SP2330-60m	Helium	35 psi head pressure
3	Finnigan 4021	SP2330-60m	Helium	29 cm/sec
4	Finnigan 4000 (INCOS data system)	SP2330-60m	Helium	21 cm/sec
5	Finnigan 4021	SP2330-60m	Helium	25 cm/sec
6	Finnigan 4023	SP2340-60m	Helium	30 cm/sec
7	Hewlett-Packard 5985A	SP2340-50m	Helium	14 cm/sec
8	Finnigan OWA-30	SP2330-60m	Hydrogen	49 cm/sec
9	Finnigan 4000	SP2340-50m	Helium	30 cm/sec
10	Finnigan 4021	SP2330-60m	Helium	∿60 cm/sec (30 psi head pressure)
11	Varian Mat 311A	SP2330-60m	Helium	25 cm/sec

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APPENDIX A

2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN METHOD 613

United States
Environmental Protection
Agency

Environmental Monitoring and
Support Laboratory
Cincinnati OH 45268

Research and Development

600/4-82-057

EPA

Test Method

2,3,7,8-Tetrachlorodibenzo-p-Dioxin — Method 613

1. Scope and Application

1.1 This method covers the determination of 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD). The following parameter may be determined by this method:

Parameter	STORET No.	CAS No.
2,3,7,8-TCDD	34675	1746-01-6

1.2 This is a gas chromatographic/mass spectrometer (GC/MS) method applicable to the determination of 2,3,7,8-TCDD in municipal and industrial discharges as provided under 40 CFR 136.1. Method 625 may be used to screen samples for 2,3,7,8-TCDD. When the screening test is positive, the final qualitative confirmation and quantification must be made using method 613.

1.3 The method detection limit (MDL, defined in Section 14.1)[1] for 2,3,7,8-TCDD is listed in Table 1. The MDL for a specific wastewater may be different depending upon the nature of interferences in the sample matrix.

1.4 Because of the extreme toxicity of this compound, the analyst must prevent exposure to himself, or to others, by materials known or believed to contain 2,3,7,8-TCDD. Section 4 of this method contains guidelines and protocols that serve as minimum safe-handling standards in a limited access laboratory.

1.5 Any modification of this method, beyond those expressly permitted, shall be considered as major modifications subject to application and approval of alternate test procedures under 40 CFR 136.4 and 136.5.

1.6 This method is restricted to use only by or under the supervision of analysts experienced in the use of gas chromatograph/mass spectrometers and skilled in the interpretation of mass spectra. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.

2. Summary of Method

2.1 A one-liter sample of wastewater is spiked with an internal standard of labeled 2,3,7,8-TCDD. The spiked sample is then extracted with methylene chloride using separatory funnel techniques. The extract is concentrated and exchanged to hexane while being concentrated to a volume of 1.0 mL or less. Capillary column GC/MS conditions are described which allow for the separation and measurement of 2,3,7,8-TCDD in the extract[2,3].

2.2 The method provides selected column chromatographic cleanup procedures to aid in the elimination of interferences that may be encountered.

3. Interferences

3.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample

processing hardware that lead to discrete artifacts and/or elevated backgrounds at the ions monitored. All of these materials must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5.

3.1.1 Glassware must be scrupulously cleaned.[4] Clean all glassware as soon as possible after use by rinsing with the last solvent used in it. This should be followed by detergent washing with hot water, and rinses with tap water and distilled water. Glassware should then be drained dry, and heated in a muffle furnace at 400 °C for 15 to 30 minutes. Some thermally stable materials, such as PCBs, may not be eliminated by this treatment. Solvent rinses with acetone and pesticide quality hexane may be substituted for the muffle furnace heating. Volumetric ware should not be heated in a muffle furnace. After drying and cooling, glassware should be sealed and stored in a clean environment to prevent any accumulation of dust or other contaminants. Store it inverted or capped with aluminum foil.

3.1.2 The use of high purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

3.2 Matrix interferences may be caused by contaminants that are co-extracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality being sampled. 2,3,7,8-TCDD is often associated with other interfering chlorinated compounds which are at concentrations several magnitudes higher than that of 2,3,7,8-TCDD. The cleanup procedures in Section 11 can be used to overcome many of these interferences, but unique samples may require additional cleanup approaches[1,5-7] to eliminate false positives and achieve the method detection limit listed in Table 1.

3.3 The primary column, SILAR-10C, resolves 2,3,7,8-TCDD from the other 21 isomers[3]. Positive results obtained using any other gas chromatographic column must be confirmed using this column.

4. Safety

4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are identified[8-10]. Benzene and 2,3,7,8-TCDD have been identified as suspected human or mammalian carcinogens.

4.2 Each laboratory must develop a strict safety program for handling of 2,3,7,8-TCDD. The following laboratory practices are recommended:

4.2.1 Contamination of the laboratory will be minimized by conducting all manipulations in a hood.

4.2.2 The effluents of sample splitters for the gas chromatograph and roughing pumps on the GC/MS should pass through either a column of activated charcoal or be bubbled through a trap containing oil or high-boiling alcohols.

4.2.3 Liquid waste should be dissolved in methanol or ethanol and irradiated with ultraviolet light with wavelength greater than 290 nm for several days. (Use F 40 BL lamps or equivalent.) Analyze liquid wastes and dispose of the solutions when 2,3,7,8-TCDD can no longer be detected.

4.3 Dow Chemical U.S.A. has issued the following precautions (revised 11/78) for safe handling of 2,3,7,8-TCDD in the laboratory:

4.3.1 The following statements on safe handling are as complete as possible on the basis of available toxicological information. The precautions for safe handling and use are necessarily general in nature since detailed, specific recommendations can be made only for the particular exposure and circumstances of each individual use. Inquiries about specific operations or uses may be addressed to the Dow Chemical Company. Assistance in evaluating the health hazards of particular plant conditions may be obtained from certain consulting laboratories and from State Departments of Health or of Labor, many of which have an industrial health service. 2,3,7,8-TCDD is extremely toxic to laboratory animals. However, it has been handled for years without injury in analytical and biological laboratories. Techniques used in handling radioactive and infectious materials are applicable to 2,3,7,8-TCDD.

4.3.1.1 Protective Equipment: Throw-away plastic gloves, apron or lab coat, safety glasses and lab hood adequate for radioactive work.

4.3.1.2 Training: Workers must be trained in the proper method of removing of contaminated gloves and clothing without contacting the exterior surfaces.

4.3.1.3 Personal Hygiene: Thorough washing of hands and forearms after each manipulation and before breaks (coffee, lunch, and shift).

4.3.1.4 Confinement: Isolated work area, posted with signs, segregated glassware and tools, plastic-backed absorbent paper on benchtops.

4.3.1.5 Waste: Good technique includes minimizing contaminated waste. Plastic bag liners should be used in waste cans. Janitors must be trained in safe handling of waste.

4.3.1.6 Disposal of Wastes: 2,3,7,8-TCDD decomposes above 800 °C. Low-level waste such as the absorbent paper, tissues, animal remains and plastic glvoes may be burned in a good incinerator. Gross quantities (milligrams) should be packaged securely and disposed through commercial or governmental channels which are capable of handling high-level radioactive wastes or extremely toxic wastes. Liquids should be allowed to evaporate in a good hood and in a disposable container. Residues may then be handled as above.

4.3.1.7 Decontamination: Personal—any mild soap with plenty of scrubbing action: Glassware, Tools, and Surfaces—Chlorothene NU Solvent (Trademark of the Dow Chemical Company) is the least toxic solvent shown to be effective. Satisfactory cleaning may be accomplished by rinsing with Chlorothene, then washing with any detergent and water. Dish water may be disposed to the sewer. It is prudent to minimize solvent wastes because they may require special disposal through commercial sources which are expensive.

4.3.1.8 Laundry: Clothing known to be contaminated should be disposed with the precautions described under "Disposal of Wastes." Lab coats or other clothing worn in 2,3,7,8-TCDD

work area may be laundered. Clothing should be collected in plastic bags. Persons who convey the bags and launder the clothing should be advised of the hazard and trained in proper handling. The clothing may be put into a washer without contact if the launderer knows the problem. The washer should be run through a cycle before being used again for other clothing.

4.3.1.9 Wipe Tests: A useful method of determining cleanliness of work surfaces and tool is to wipe the surface with a piece of filter paper. Extraction and analysis by gas chromatography can achieve a limit of sensitivity of 0.1 μg per wipe. Less than 1 μg 2,3,7,8-TCDD per sample indicates acceptable cleanliness; anything higher warrants further cleaning. More than 10 μg on a wipe sample indicates an acute hazard and requires prompt cleaning before further use of the equipment or work space and indicates further that unacceptable work practices have been employed in the past.

4.3.1.10 Inhalation: Any procedure that may produce airborne contamination must be done with good ventilation. Gross losses to a ventilation system must not be allowed. Handling of the dilute solutions normally used in analytical and animal work presents no inhalation hazards except in case of an accident.

4.3.1.11 Accidents: Remove contaminated clothing immediately, taking precautions not to contaminate skin or other articles. Wash exposed skin vigorously and repeatedly until medical attention is obtained.

5. Apparatus and Materials

5.1 Sampling equipment, for discrete or composite sampling.

5.1.1 Grab sample bottle—Amber glass, one-liter or one-quart volume, fitted with screw caps lined with Teflon. Foil may be substituted for Teflon if the sample is not corrosive. If amber bottles are not available, protect samples from light. The container must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.

5.1.2 Automatic sampler (optional)—Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4 °C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing should be thoroughly rinsed with methanol, followed by repeated rinsings with distilled water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow proportional composites.

5.1.3 Clearly label all samples as "POISON" and ship according to U.S.D.O.T. requirements.

5.2 Glassware (All specifications are suggested. Catalog numbers are included for illustration only).

5.2.1 Separatory funnel—2000-mL and 125-mL, with Teflon stopcock.

5.2.2 Concentrator tube, Kuderna-Danish—10-mL, graduated (Kontes K-570050-1025 or equivalent). Calibration must be checked at the volumes employed in the test. Ground glass stopper is used to prevent evaporation of extracts.

5.2.3 Evaporative flask, Kuderna-Danish—500-mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs.

5.2.4 Snyder column, Kuderna-Danish—three-ball macro (Kontes K-503000-0121 or equivalent).

5.2.5 Snyder column, Kuderna-Danish—two-ball micro (Kontes K-569001-0219 or equivalent).

5.2.6 Vials—Amber glass, 10- to 15-mL capacity, with Teflon-lined screw cap.

5.2.7 Chromatography column—300 mm long × 10 mm ID with coarse fritted disc at bottom and Teflon stopcock.

5.2.8 Chromatography column—400 mm long × 11 mm ID with coarse fritted disc at bottom and Teflon stopcock.

5.3 Boiling chips—approximately 10/40 mesh. Heat to 400 °C for 30 minutes or Soxhlet extract with methylene chloride.

5.4 Water bath—Heated, with concentric ring cover, capable of temperature control (± 2 °C). The bath should be used in a hood.

5.5 GC/MS system.

5.5.1 Gas chromatograph—An analytical system complete with all required accessories including syringes, analytical columns, and gases. The injection port must be designed for capillary columns. Either split, splitless, or on-column injection techniques may be employed, as long as the requirements of Section 7.1.1 are achieved.

5.5.2 Primary column—50 m long × 0.25 mm ID glass, coated with SILAR-10C (or equivalent). An equivalent column must resolve 2,3,7,8-TCDD from the other 21 TCDD isomers. Guidelines for the use of alternate columns are provided in Section 12.1.

5.5.3 Mass Spectrometer—Either low resolution mass spectrometers (LRMS) or high resolution mass spectrometers (HRMS) may be used. The mass spectrometer must be equipped with a 70 volt (nominal) ion source and be capable of acquiring ion abundance data in real time Selected Ion Monitoring (SIM) for groups of four or more ions.

5.5.4 GC/MS interface—Any gas chromatograph to mass spectrometer interface can be used that achieves the requirements of Section 7.1.1 constructed of glass or glass-lined materials are recommended. Glass surfaces can be deactivated by silanizing with dichlorodimethylsilane. To achieve maximum sensitivity, the exit end of the capillary column should be placed in the ion source. A short piece of fused silica capillary can be used as the interface to overcome problems associated with straightening the exit end of glass capillary columns.

5.5.5 The SIM data acquired during the chromatographic program is defined as the Selected Ion Current Profile (SICP). The SICP can be acquired under computer control or as real time analog output. If computer control is used, there must be software available to plot the SICP and report peak height or area data for any ion in the SICP between specified time or scan number limits.

5.6 Balance—Analytical, capable of accurately weighing 0.0001g.

6. Reagents

6.1 Reagent water—Reagent water is defined as a water in which an interferent is not observed at the MDL of 2,3,7,8-TCDD.

6.2 Sodium hydroxide solution—(ACS) Dissolve 400 g NaOH in reagent water and dilute to one liter. Wash the solution with methylene chloride and with hexane before use.

6.3 Sodium thiosulfate—(ACS) Granular.

6.4 Sulfuric acid (Conc.)—(ACS) sp. gr. 1.84.

6.5 Methylene chloride, hexane, benzene, tetradecane—Pesticide quality or equivalent.

6.6 Sodium sulfate—(ACS) Granular, anhydrous (purified by heating at 400 °C for four hours in a shallow tray).

6.7 Alumina—neutral, 80/200 mesh (Fisher Scientific Co., No. A-540 or equivalent). Before use, activate for 24 hours at 130 °C in a foil covered glass container.

6.8 Silica gel—high purity grade, 100/120 mesh, (Fisher Scientific Co., No. S-679 or equivalent).

6.9 Stock standard solutions (1.00 µg/µL)—Stock standard solutions can be prepared from pure standard materials or purchased as certified solutions. Acetone should be used as the solvent for spiking solutions; ortho-xylene is recommended for calibration standards for split injectors; and tetradecane is recommended for splitless or on-column injectors. Analyze stock internal standards to verify the absence of native 2,3,7,8-TCDD.

6.9.1 Prepare stock standard solutions of 2,3,7,8-TCDD (mol wt 320) and either $^{37}Cl_4$ 2,3,7,8-TCDD (mol wt 328) or $^{13}C_{12}$ 2,3,7,8-TCDD (mol wt 332) in an isolated area by accurately weighing about 0.0100 g of pure material. Dissolve the material in pesticide quality solvent, dilute to volume in a 10-mL volumetric flask. If compound purity is certified at 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards can be used at any concentration if they are certified by the manufacturer or by an independent source.

6.9.2 Transfer the stock standard solutions into Teflon-sealed screw-cap bottles. Store in an isolated refrigerator protected from light. Stock standard solutions should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards or spiking solutions from them.

6.9.3 Stock standard solutions must be replaced after six months or sooner if comparison with check standards indicates a problem.

6.10 Internal standard spiking solutions (25 ng/mL)—Using stock standard solution. Prepare a spiking solution in acetone of either $^{13}C_{12}$ or $^{37}Cl_4$ 2,3,7,8-TCDD at a concentration of 25 ng/mL. (See Section 10.2.1.)

7. Calibration

7.1 Establish gas chromatographic conditions for the GC/MS system equivalent to those indicated in Table 1 and SIM conditions for the mass spectrometer as described in Section 12.2. The GC/MS system must be calibrated using the internal standard technique. By injecting calibration standards, establish ion response factors for 2,3,7,8-TCDD vs. an internal standard (either $^{13}C_{12}$ or $^{37}Cl_4$ 2,3,7,8-TCDD).

7.1.1 Using stock standards, prepare GC/MS calibration standard solutions that will allow measurement of relative response factors of at least three concentration ratios of 2,3,7,8-TCDD to internal standard. Each solution must be prepared to contain the internal standard at a concentration of 25 ng/mL. If any interferences are contributed by the internal standard at m/e 320 and 322, its concentration may be reduced in calibration standards and in the internal spiking solution (Section 6.10). One of the calibration standard solutions should be prepared to contain 2,3,7,8-TCDD representing a concentration near, but above, the MDL. The other 2,3,7,8-TCDD concentrations should correspond to the expected range of concentrations found in real samples.

7.1.2 Using injections of 2 to 5 µL, tabulate peak height or area response against the concentration of 2,3,7,8-TCDD and internal standard in each calibration standard, and calculate response factors (RF) for 2,3,7,8-TCDD using equation 1.

$$\text{Eq. 1} \quad RF = (A_s C_{is})/(A_{is} C_s)$$

where:

A_s = SIM response for 2,3,7,8-TCDD m/e 320.

A_{is} = SIM response for internal standard, m/e 332 for $^{13}C_{12}$ 2,3,7,8-TCDD, m/e 328 for $^{37}Cl_4$ 2,3,7,8-TCDD.

C_{is} = Concentration of the internal standard, (µg/L).

C_s = Concentration of 2,3,7,8-TCDD (µg/L).

If the RF value over the working range is a constant (<10% RSD), the RF can be assumed to be invariant and the average RF can be used for calculations. Alternatively, the results can be used to plot a calibration curve of response ratios, A_s/A_{is}, vs. RF.

7.1.3 The working calibration curve or RF must be verified on each working day by the measurement of one or more 2,3,7,8-TCDD calibration standards. If the response for 2,3,7,8-TCDD varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared.

7.2 Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interferences from the reagents.

8. Quality Control

8.1 Each laboratory that uses this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated. Ongoing performance checks must be compared with established performance criteria to determine if the results of analyses are within accuracy and precision limits expected of the method.

8.1.1 Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.

8.1.2 In recognition of the rapid advances that are occurring in both mass spectrometry and gas chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications are made to the method, the analyst is required to repeat the procedure in Section 8.2.

8.1.3 The laboratory must spike and analyze a minimum of 10% of all samples with native 2,3,7,8-TCDD to monitor continuing laboratory performance. This procedure is described in Section 8.4.

8.2 To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.

8.2.1 Using stock standard solution, prepare a quality control check sample concentrate containing 2,3,7,8-TCDD at a concentration of 15 ng/mL in acetone.

8.2.2 Using a pipet, add 1.00 mL of the check sample concentrate to each of a minimum of four 1000-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.

8.2.3 Calculate the average percent recovery, (R), and the standard deviation of the percent recovery (s), for the results. Wastewater background corrections must be made before R and s calculations are performed.

8.2.4 Using Table 2, note the average recovery (X) and standard deviation (p) expected for each method parameter. Compare these to the calculated values for R and s. If $s > 2p$ or $|X - R| > 2p$, review potential problem areas and repeat the test.

8.2.5 The U.S. Environmental Protection Agency plans to establish performance criteria for R and s based upon the result of interlaboratory testing. When they become available, these criteria must be met before any samples may be analyzed.

8.3 The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.

8.3.1 Calculate upper and lower control limits for method performance:

Upper Control Limit (UCL) = R + 3s
Lower Control Limit (LCL) = R − 3s

where R and s are calculated as in Section 8.2.3. The UCL and LCL can be used to construct control charts[11] that are useful in observing trends in performance. The control limits above must be replaced by method performance criteria as they become available from the U.S. Environmental Protection Agency.

8.3.2 The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as R ± s. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s. Alternately, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly[10].

8.4 The laboratory is required to collect a portion of their samples in duplicate to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery for 2,3,7,8-TCDD does not fall within the control limits for method performance, the results reported for 2,3,7,8-TCDD in all samples processed as part of the same set must be qualified as described in Section 13.4. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.

8.5 Before processing any samples, the analyst should demonstrate through the analysis of a one-liter aliquot of reagent water, that all glassware and reagent interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a laboratory reagent blank should be processed as a safeguard against laboratory contamination.

8.6 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Field duplicates may be analyzed to monitor the precision of the sampling technique. Whenever possible, the laboratory should perform analysis of standard reference materials and participate in relevant performance evaluation studies.

9. Sample Collection, Preservation, and Handling

9.1 Grab samples must be collected in glass containers. Conventional sampling practices[12] should be followed, except that the bottle must not be prewashed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of Tygon and other potential sources of contamination.

9.2 The samples must be iced or refrigerated at 4 °C and protected from light from the time of collection until extraction. If the sample contains residual chlorine, add 80 mg of sodium thiosulfate per each liter of sample. U.S. Environmental Protection Agency methods 330.4 and 330.5 may be used for measurement of residual chlorine[13]. Field test kits are available for this purpose.

9.3 Label all samples and containers POISON and ship according to applicable U.S. Department of Transportation regulations.

9.4 All samples must be extracted within 7 days and completely analyzed within 40 days of extraction[2].

10. Sample Extraction

CAUTION: When using this method to analyze for 2,3,7,8-TCDD, all of the following operations must be performed in a limited access laboratory with the analyst wearing full protective covering for all exposed skin surfaces. See Section 4.2.

10.1 Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a two-liter separatory funnel.

10.2 Add 1.00 mL of internal standard spiking solution to the sample in the separatory funnel. If the final extract will be concentrated to a fixed volume below 1.00 mL (Section 12.3), only that volume of spiking solution should be added to the sample so that the final extract will represent 25 ng/mL at the time of analysis.

10.3 Add 60 mL methylene chloride to the sample bottle, seal, and shake 30 seconds to rinse the inner surface. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for two minutes with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, centrifugation, or other physical methods. Collect the methylene chloride extract in a 250-mL Erlenmeyer flask.

10.4 Add a second 60-mL volume of methylene chloride to the sample bottle and repeat the extraction procedure a second time, combining the extracts in the Erlenmeyer flask. Perform a third extraction in the same manner.

10.5 Assemble a Kuderna-Danish (K-D) concentrator by attaching a

10-mL concentrator tube to a 500-mL evaporative flask. Other concentration devices or techniques may be used in place of the K-D if the requirements of Section 8.2 are met.

10.6 Pour the combined extract into the K-D concentrator. Rinse the Erlenmeyer flask and column with 20 to 30 mL of methylene chloride to complete the quantitative transfer.

10.7 Add one or two clean boiling chips to the evaporative flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL methylene chloride to the top. Place the K-D apparatus on a hot water bath (60 to 65 °C) so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation the balls of the column will actively chatter but the chambers will not flood with condensed solvent. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus from the water bath and allow it to drain and cool for at least 10 minutes.

10.8 Momentarily remove the Snyder column, add 50 mL hexane and a new boiling chip and replace the column. Raise the temperature of the water bath to 85 to 90 °C. Concentrate the extract as in Section 10.7, except using hexane to prewet the column. Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of hexane. A 5-mL syringe is recommended for this operation. Set aside a K-D glassware for reuse in Section 10.14.

10.9 Pour the hexane from the concentrator tube into a 125-mL separatory funnel. Rinse the concentrator tube four times with 10-mL aliquots of hexane. Combine all rinses in the 125-mL separatory funnel.

10.10 Add 50 mL of 10N sodium hydroxide solution to the funnel and shake for 30 to 60 seconds. Discard the aqueous phase.

10.11 Perform a second wash of the organic layer with 50 mL of reagent water. Discard the aqueous phase.

10.12 Wash the hexane layer with at least two 50-mL aliquots of concentrated sulfuric acid. Continue washing the hexane layer with 50-mL aliquots of concentrated sulfuric acid until the acid layer remains colorless. Discard all acid fractions.

10.13 Wash the hexane layer with two 50-mL aliquots of reagent water. Discard the aqueous phases.

10.14 Transfer the hexane layer into a 125-mL Erlenmeyer flask containing 1 to 2 g anhydrous sodium sulfate. Swirl the flask for 30 seconds and decant the hexane into the reassembled K-D apparatus. Complete the quantitative transfer with two 10-mL hexane rinses of the Erlenmeyer flask.

10.15 Replace the one or two clean boiling chips and concentrate the extract to 6 to 10 mL as described in Section 10.8.

10.16 Add a clean boiling chip and attach a micro-Snyder column. Prewet the column by adding about 1 mL hexane to the top. Place the K-D apparatus on the 80 °C water bath so that the concentrator tube is partially immersed in the hot water. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 5 to 10 minutes. At the proper rate of distillation the balls of the column will actively chatter but the chambers will not flood. When the apparent volume of liquid reaches about 0.5 mL, remove the K-D apparatus and allow it to drain for at least 10 minutes while cooling. Remove the micro Snyder column and rinse its lower joint into the concentrator tube with 0.2 mL hexane. Adjust the extract volume to 1.0 mL with hexane. Stopper the concentrator tube and store refrigerated and protected from light if GC/MS analysis or cleanup will not be performed immediately.

10.17 Determine the original sample volume by refilling the sample bottle to the mark with water and measuring the volume in a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11. Cleanup and Separation

11.1 Cleanup procedures may not be necessary for a relatively clean sample matrix. The cleanup procedures recommended in this method have been used for the analysis of various clean waters and industrial effluents. The single operator precision and accuracy data in Table 2 was gathered using the recommended cleanup procedures. If particular circumstances demand the use of an alternative cleanup procedure, the analyst must determine the elution profile and demonstrate that the recovery of 2,3,7,8-TCDD is reproducible and equivalent to the recovery of the internal standard. Two cleanup column options are offered to the analyst in this section. The alumina column should be used first to overcome interferences. If background problems are still encountered, the silica gel column may be helpful. Other cleanup procedures have been described to overcome special interference problems[1,5-7].

11.2 Alumina column cleanup for 2,3,7,8-TCDD.

11.2.1 Fill a 300 mm long × 10 mm ID chromatography column with activated alumina to the 150 mm level, tapping the column gently to settle the alumina. Add 10 mm anhydrous sodium sulfate to the top of the alumina.

11.2.2 Preelute the column with 50 mL hexane. Adjust the elution rate to 1 mL/min. Discard the eluate and just prior to exposure of the sodium sulfate layer to the air, transfer the entire 1.0 mL sample extract onto the column, using two additional 2-mL portions of hexane to complete the transfer.

11.2.3 Just prior to exposure of the sodium sulfate layer to the air, add 50 mL 3% methylene chloride/97% hexane (V/V) and continue the elution of the column. Discard the eluate.

11.2.4 Next elute the column with 50 mL 20% methylene chloride/80% hexane (V/V) into a 500 mL K-D flask equipped with a 10-mL concentrator tube. Concentrate the collected fraction to 1.0 mL by standard K-D techniques and analyze by GC/MS.

11.3 Silica gel column cleanup for 2,3,7,8-TCDD.

11.3.1 Fill a 400 mm long × 11 mm ID chromatography column with silica gel to the 300 mm level, tapping the column gently to settle the silica gel. Add 10 mm anhydrous sodium sulfate to the top of the silica gel.

11.3.2 Preelute the column with 50 mL 20% benzene/80% hexane (V/V). Adjust the elution rate to 1 mL/min. Discard the eluate and just prior to exposure of the sodium sulfate layer to the air, transfer the entire 1.0 mL sample extract onto the column, using two 2 mL portions of 20% benzene/80% hexane to complete the transfer.

11.3.3 Just prior to exposure of the sodium sulfate layer to the air, add 40 mL 20% benzene/80% hexane to the column. Collect the eluate in a clean

500-mL K-D flask equipped with a 10-mL concentrator tube.

11.3.4 Evaporate the fraction to 1.0 mL by standard K-D techniques and analyze by GC/MS.

12. GC/MS Analysis

12.1 Table 1 summarizes the recommended gas chromatographic capillary column and operating conditions for the instrument. Included in this table are the estimated retention time and MDL that can be achieved by this method. Other capillary columns or chromatographic conditions may be used to screen samples if the requirements of Section 8.2 are met. Confirmation of 2,3,7,8-TCDD must be accomplished using a GC column that separates 2,3,7,8-TCDD from all other TCDD isomers.

12.2 Analyze standards and samples with the mass spectrometer operating in the SIM mode using a dwell time to give at least seven points per peak. For LRMS, use ions at m/e 320, 322, and 257 for 2,3,7,8-TCDD and either the ion at m/e 328 for ^{37}Cl 2,3,7,8-TCDD or m/e 332 for ^{13}C 2,3,7,8-TCDD. For HRMS, use ions at m/e 319.8965 and 321.8936 for 2,3,7,8-TCDD and either the ion at m/e 327.8847 for ^{37}Cl 2,3,7,8-TCDD or m/e 331.9367 for ^{13}C 2,3,7,8-TCDD.

12.3 If lower detection limits are required, the extract may be carefully evaporated to dryness under a gentle stream of nitrogen with the concentrator tube in a water bath at about 40 °C. Do this immediately before GC/MS analysis. Redissolve the extract in the desired final volume of ortho-xylene or tetradecane. The method performance data reported in Section 14 was gathered using a final extract volume of 0.2 mL.

12.4 Calibrate the system daily as described in Section 7.1.3. The volume of calibration standard injected must be measured, or be the same as all sample injection volumes.

12.5 Inject a 2 to 5 µL aliquot of the sample extract.

12.6 The presence of 2,3,7,8-TCDD is qualitatively confirmed if all of the following criteria are achieved.

12.6.1 The gas chromatographic column must resolve 2,3,7,8-TCDD from the other 21 TCDD isomers.

12.6.2 The ions for native 2,3,7,8-TCDD (LRMS-m/e 320, 322, and 257 and HRMS-m/e 320 and 322) and labeled 2,3,7,8-TCDD (m/e 328 or 332) must exhibit a simultaneous maximum at a retention time that matches that of native 2,3,7,8-TCDD in the calibration standard, within the performance specifications of the analytical system.

12.6.3 The chlorine isotope ratio at m/e 320 and m/e 322 must agree to within ±10% of that in the calibration standard.

12.6.4 The signal of all peaks must be greater than 2.5 times the noise level.

12.7 For quantitation, measure the response of the m/e 320 peak for 2,3,7,8-TCDD and the m/e 332 peak for $^{13}C_{12}$ 2,3,7,8-TCDD or the m/e 328 peak for $^{37}Cl_4$ 2,3,7,8-TCDD.

12.8 Co-eluting impurities are suspected if all criteria are achieved except those in Section 12.6.3. In this case, another SIM analysis using ions at m/e 257, 259, 320 and either m/e 328 or m/e 322 can be performed. The ions at m/e 257 and m/e 259 are indicative of the loss of one chlorine and one carbonyl group from 2,3,7,8-TCDD. If the ions m/e 257 and m/e 259 give a chlorine isotope ratio that agrees to within ±10% of the same cluster in the calibration standards, than the presence of TCDD can be confirmed. Co-eluting DDD, DDE, and PCB residues can be confirmed, but will require another injection using the appropriate SIM ions or full repetitive mass scans. If the response for ^{37}Cl 2,3,7,8-TCDD at m/e 328 is too large, PCB contamination is suspected and can be confirmed by examining the response at both m/e 326 and m/e 328. The ^{37}Cl 2,3,7,8-TCDD internal standard gives negligible response at m/e 326. These pesticide residues can be removed using the alumina column cleanup.

12.9 If broad background interference restricts the sensitivity of the GC/MS analysis, the analyst should employ additional cleanup procedures and reanalyze by GC/MS.

12.10 In those circumstances where these procedures do not yield a definitive conclusion, then the use of high resolution mass spectrometry is suggested.[5]

13. Calculations

13.1 Calculate the concentration of 2,3,7,8-TCDD in the sample using the response factor (RF) determined in 7.1.2 and equation 2.

Eq. 2

$$\text{Concentration, } \mu g/l = \frac{(A_s)(I_s)}{(A_{is})(RF)(V_o)}$$

where:

A_s = SIM response for 2,3,7,8-TCDD ion at m/e 320.

A_{is} = SIM response for the internal standard ion at m/e 328 or 332.

I_s = Amount of internal standard added to each extract (µg).

V_o = Volume of water extracted, in liters.

13.2 For each sample, calculate the percent recovery of the internal standard by comparing the area of the ion peak measured in the sample to the area of the same peak in the calibration standard. If the recovery is below 50%, the results of the sample analysis must be qualified as described in Section 13.4.

13.3 Report results in micrograms per liter. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.

13.4 For samples processed as part of a set where the laboratory spiked sample recovery falls outside of the control limits in Section 8.3 or the internal standard recovery is below 50%, the data for 2,3,7,8-TCDD must be labeled as suspect.

14. Method Performance

14.1 Method detection limit—The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero[1]. The MDL concentration listed in Table 1 was obtained using reagent water[14].

14.2 In a single laboratory (Monsanto Research Corporation), using spiked samples, the average recovery presented in Table 2 was obtained.[14] The average standard deviation of the percent recovery is also included in Table 2. The results for quality control checks for this study are presented in Table 3.

14.3 The U.S. Environmental Protection Agency is in the process of conducting an interlaboratory method study to fully define the performance of this method.

References

1. See Appendix A.

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14. "Determination of Method Detection Limits for EPA Method 613," Special letter report for EPA Contract 68-03-2863, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268.

Table 1. *Chromatographic Conditions and Method Detection Limit*

Parameter	*Retention Time (min.)*	*Detection Limit (ng/L)*
2,3,7,8-TCDD	*34.5*	*0.002*

Column conditions: SILAR-10C coated on a 50 m long 0.25 mm ID glass column with helium carrier gas at 30 cm/sec linear velocity, splitless injector. Column temperature programmed: isothermal, 100°C for 3 minutes, then programmed at 20°C/min to 180°C, and 2°C/min to 250°C.

Table 2. *Single Operator Accuracy and Precision*

Parameter	*Matrix*	*Spike µg/L*	*Average Percent Recovery*	*Standard Deviation %*
2,3,7,8-TCDD	*Reagent water*	*0.005*	*95.4*	*10.2*
2,3,7,8-TCDD	*Industrial waste*	*0.005*	*85.8*	*6.6*
2,3,7,8-TCDD	*Municipal waste*	*0.025*	*92.4*	*18.7*

Table 3. *Quality Control Results (14)*

Matrix	*320/322 Isotope Ratios*	*Internal Standard Recovery*
Reagent water	*0.79 ± 0.04*	*87%*
Industrial waste	*0.81 ± 0.01*	*104%*
Municipal waste	*0.83 ± 0.02*	*14%*

APPENDIX B

COPIES OF CHROMATOGRAMS AND INSTRUCTIONS PROVIDED TO PARTICIPATING LABORATORIES

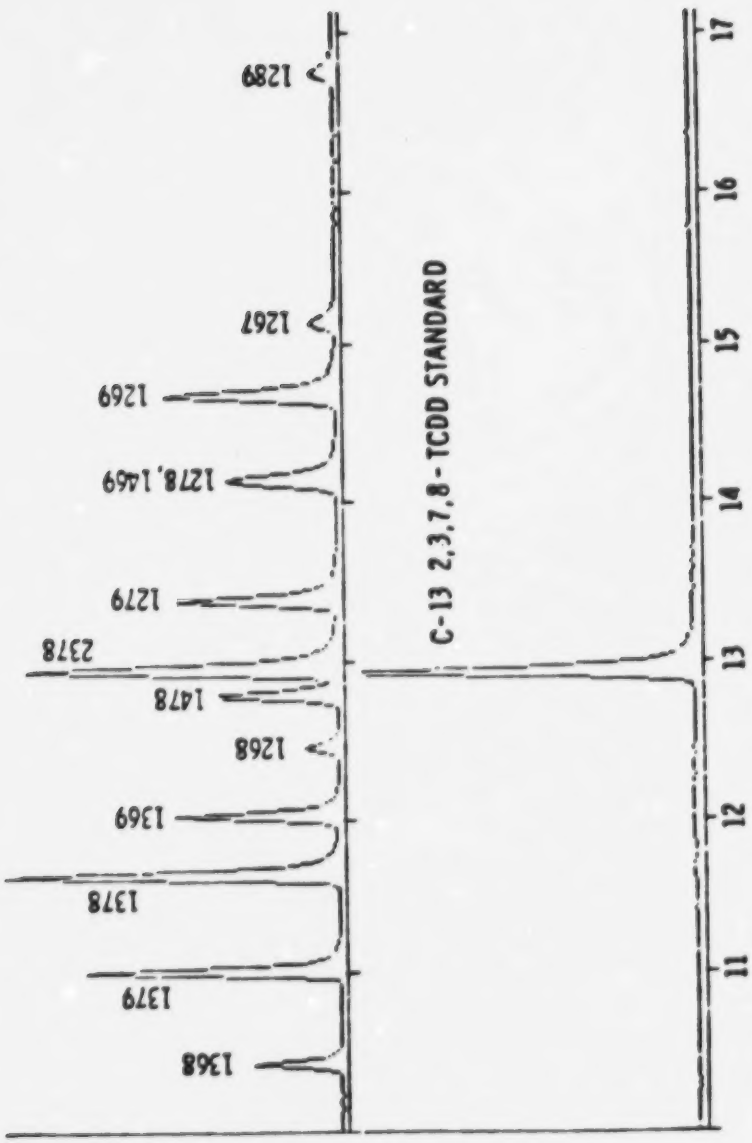


Figure 2. The tetrachlorodibenzodioxin isomers with 2:2 chlorine substitution analyzed on a glass SP-2330 column.

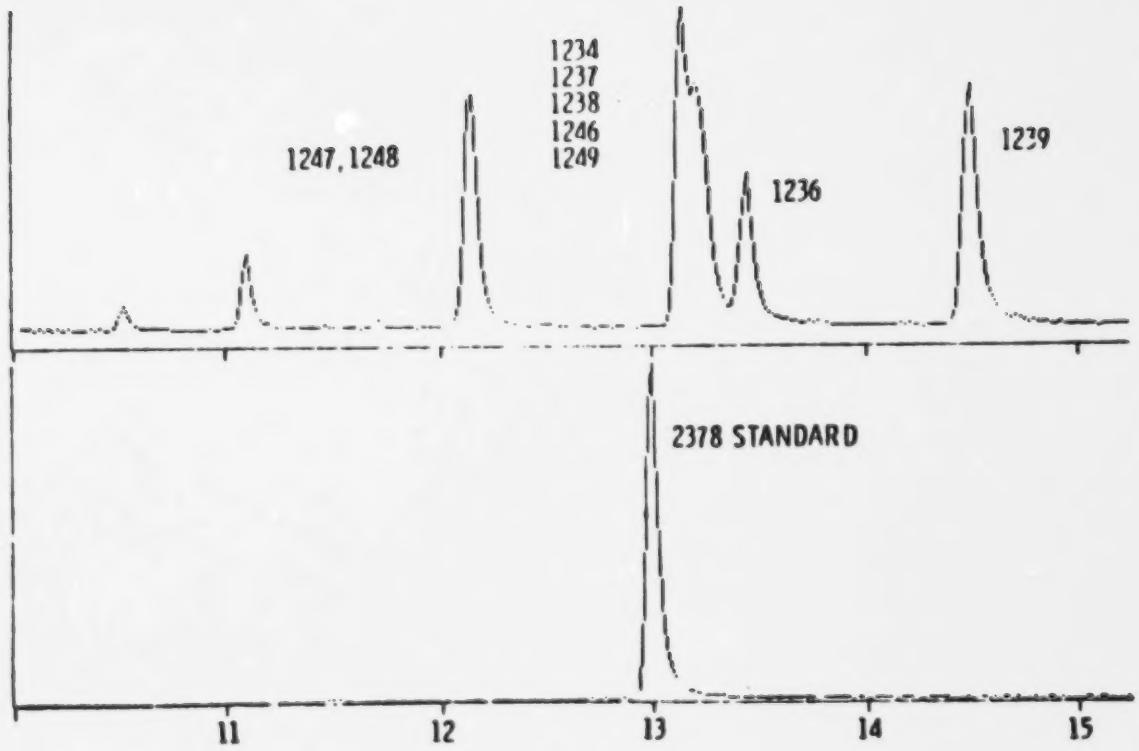


Figure 3. The tetrachlorodibenzodioxin isomers with 1:3 chlorine substitution analyzed on a glass SP-2330 column.

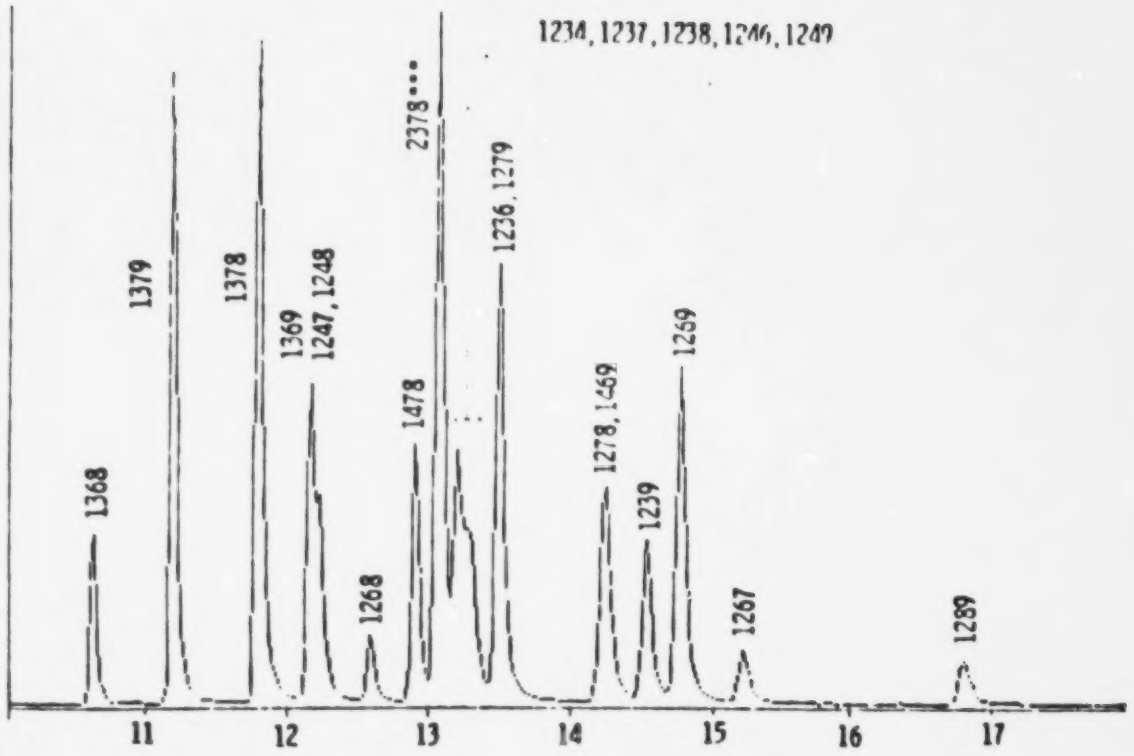


Figure 4. All 22 tetrachlorodibenzodioxin isomers analyzed on a glass SP-2330 column.

INSTRUCTIONS FOR PE SAMPLE ANALYSIS

Prepare the vials by adding the specified quantity of solvent. Recap the vials, shake and let stand several hours (TCDD dissolves very slowly in acetone and the TCDD may be deposited on the sides of the vials, even near the top).

DIOXIN PE - Add 0.5 mL (500 µL) of acetone to this vial. Four 1-liter bottles of water should each be spiked with 60 µL of this solution. Analyze each of the four samples for 2,3,7,8-TCDD using Method 613 with the alumina column cleanup. These results should be reported to MRC on the form provided. Note - there are interferring isomers present which need to be resolved in order to quantitate the 2,3,7,8-TCDD.

DIOXIN HELPER - In order to work out the chromatographic conditions for the quantitation of 2,3,7,8-TCDD in the presence of interferences, some additional sample of the same isomer mix has been provided. This can be dissolved in about 1/2 mL of tetradecane (splitless injection) or o-xylene (split injection - not recommended) as required.

DIOXIN STANDARD - This vial contains exactly 255 ng of 2,3,7,8-TCDD. A good calibration curve for the PE sample might include 20, 50, 80 ng/L equivalent concentrations.

DIOXIN ^{13}C - This vial contains exactly 700 ng of ^{13}C-2,3,7,8-TCDD. Add 0.5 mL (500 µL) acetone to the vial. Spike each PE sample with 36 µL of this solution for a 50 ppt spike (used to calculate recovery through the method).

$$\left[\frac{700 \text{ ng}}{500 \text{ µℓ}} \times 36 \text{ µℓ spike} = 50.4 \text{ ppt}\right]$$

Other Information

1. The enclosed vials are resistant to evaporation until the time that they are penetrated with a syringe. At the end of any day when the lid has been punctured, replace it with a new seal.

2. The quartz or glass injection port liner on splitless capillary injectors should be silanized. At levels of dioxins greater than 5 ppt, (all samples in the study) it is not necessary to silanize the separatory funnels.

3. Silanize with a 10% (v/v) solution of dichloridimethylsilane in toluene. Soak the pieces for about 45 minutes, rinse three times with toluene, two times with methanol, and dry in a stream of inert gas, nitrogen, or air if it is free of oil.

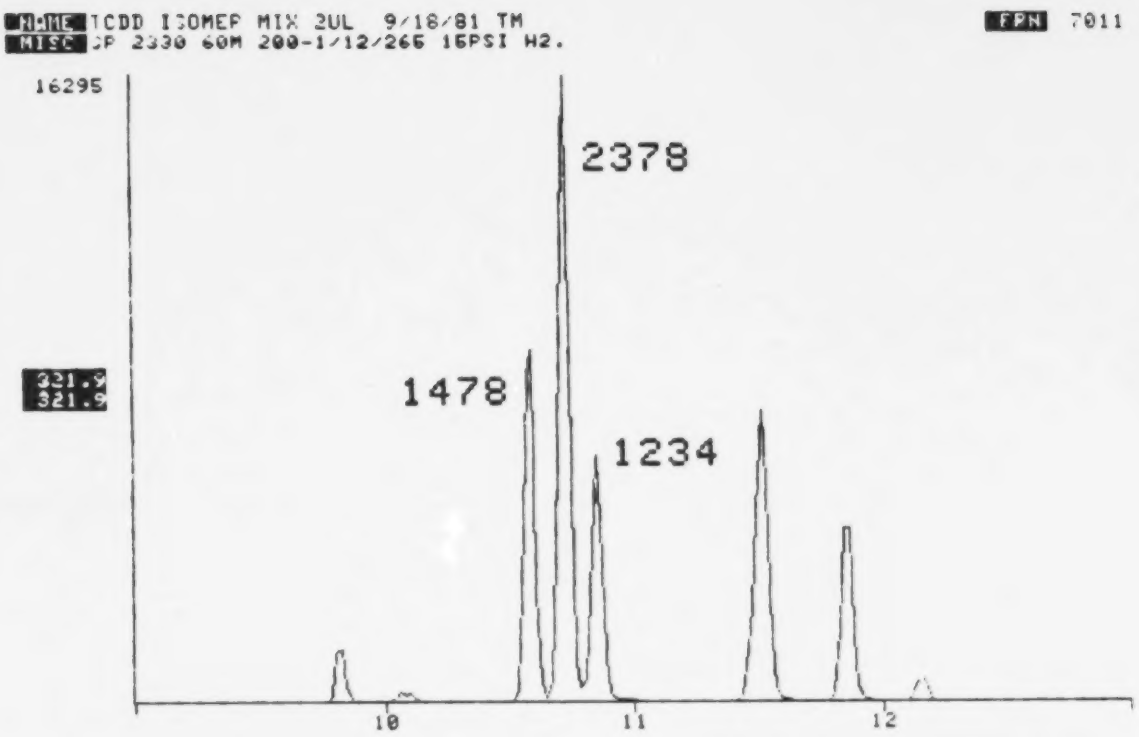
4. Silar-10C is the normal column used for 2,3,7,8-TCDD specific analyses. Recently it has been found that Supelco's SP-2330 gives even better results as can be seen in the attached spectra. Supelco's SP-2340 has not been evaluated by MRC at this time. There are no fused silica columns currently known that will separate the enclosed PE mixture.

   Additional information about the SP-2330 column can be obtained from Paul Silvas of Supelco at (814) 359-2732.

5. Combine the acid and basic wastes, neutralize with NaOH, evaporate to dryness, seal the paint can, and retain until the method study.

Chromatographs of the TCDD mixture employing both SP-2330 and SILAR 10C columns were supplied to the laboratories as presented in Figures 5 through 7. Later, newly available 60-meter fused silica SP-2330 and SP-2340 columns from Supelco were evaluated and found to give similar separation of the 2,3,7,8-TCDD from the other 21 isomers when compared to the 60-meter glass SP-2330 column. Figures 8 through 10 demonstrate the separations obtainable using these fused silica columns.

Included with the cover letter to the laboratories was a summary of health effects information on 2,3,7,8-TCDD to be read by the laboratory personnel. Also enclosed was a copy of pertinent sections of the IARC Monograph on chlorinated dioxins. It was recommended the young women of child-bearing age not be used on the project and that project personnel should use gloves and laboratory coats (disposables are best). It was recommended that particular care be exercised when combining the acid and basic wastes for disposal since the reaction is very exothermic (heat-generating). A form was included, to be signed and returned to Monsanto Research Corporation, indicating that the safety information had been read and understood.



MIXTURE OF TCDD ISOMERS. 54M SF 2330 COLUMN SUPPLIED BY SUPELCO
CONDITIONS: 15 PSI HYDROGEN.
INITIAL TEMP.: 200C. HOLD 1 MIN. RATE 130/MIN. FINAL 265C.
SYSTEM: HP 5985 GC/MS.
ANALYST: THOMAS MAZER

Figure 5. Chromatogram of TCDD mixture employing SP-2330 column.

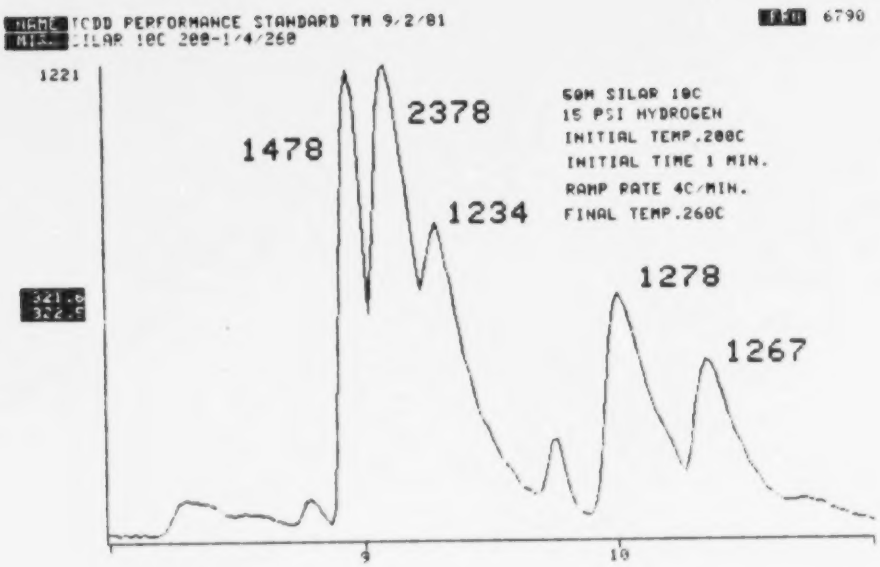


Figure 6. Chromatogram of TCDD mixture employing SILAR 10C column at 4°C/min heating rate.

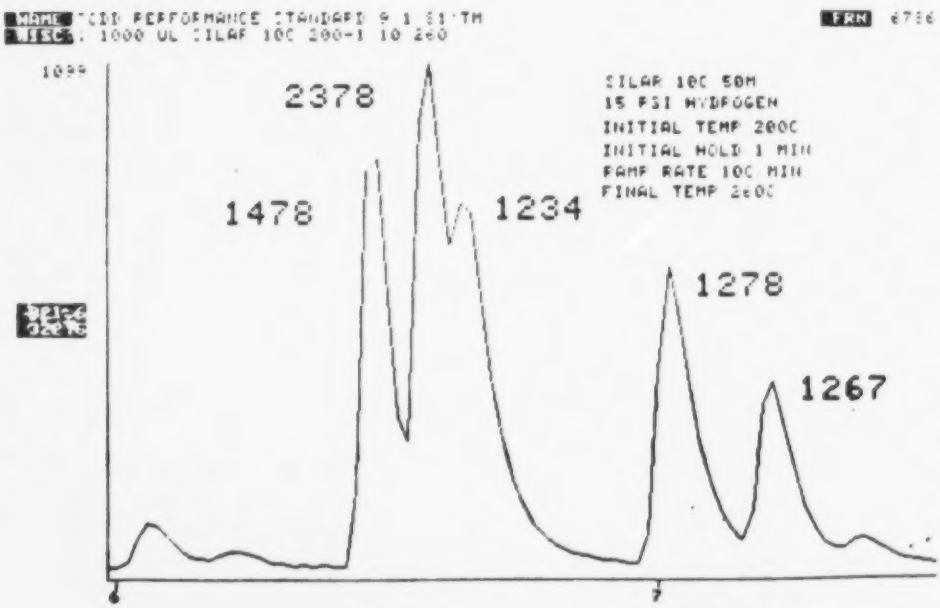


Figure 7. Chromatogram of TCDD mixture employing SILAR 10C column at 10°C/min heating rate.

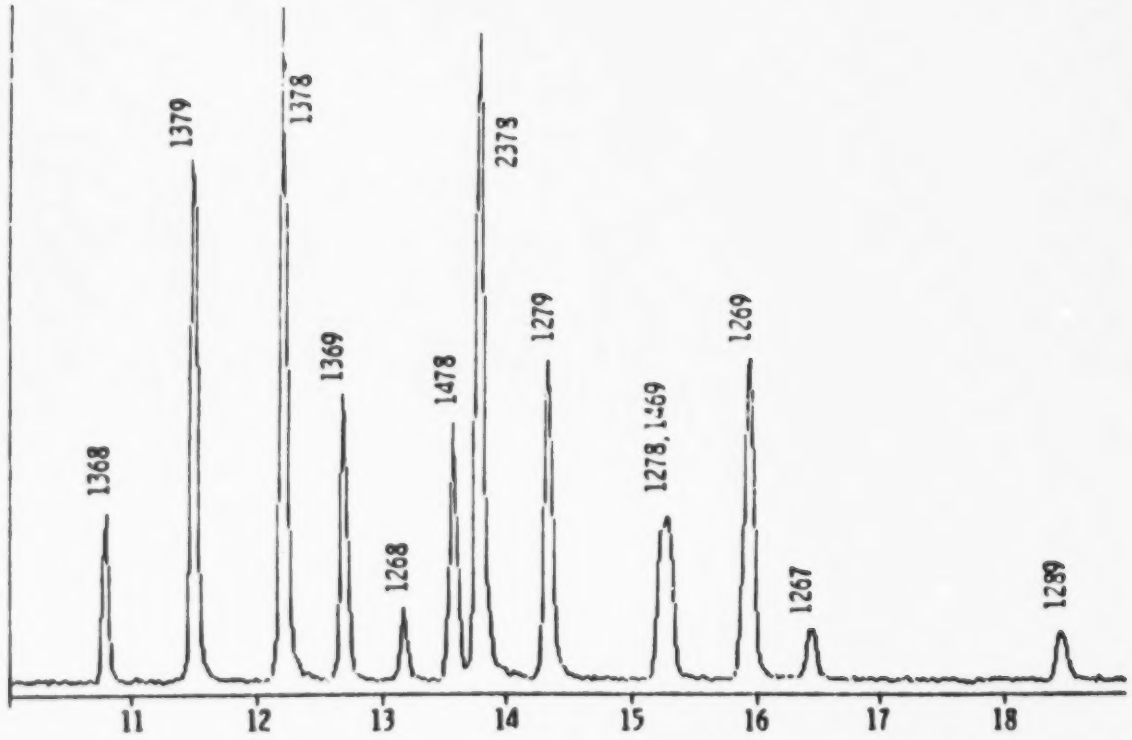


Figure 8. Tetrachlorodibenzodioxin isomers with 2:2 chlorine substitution analyzed on a fused silica SP-2340 column.

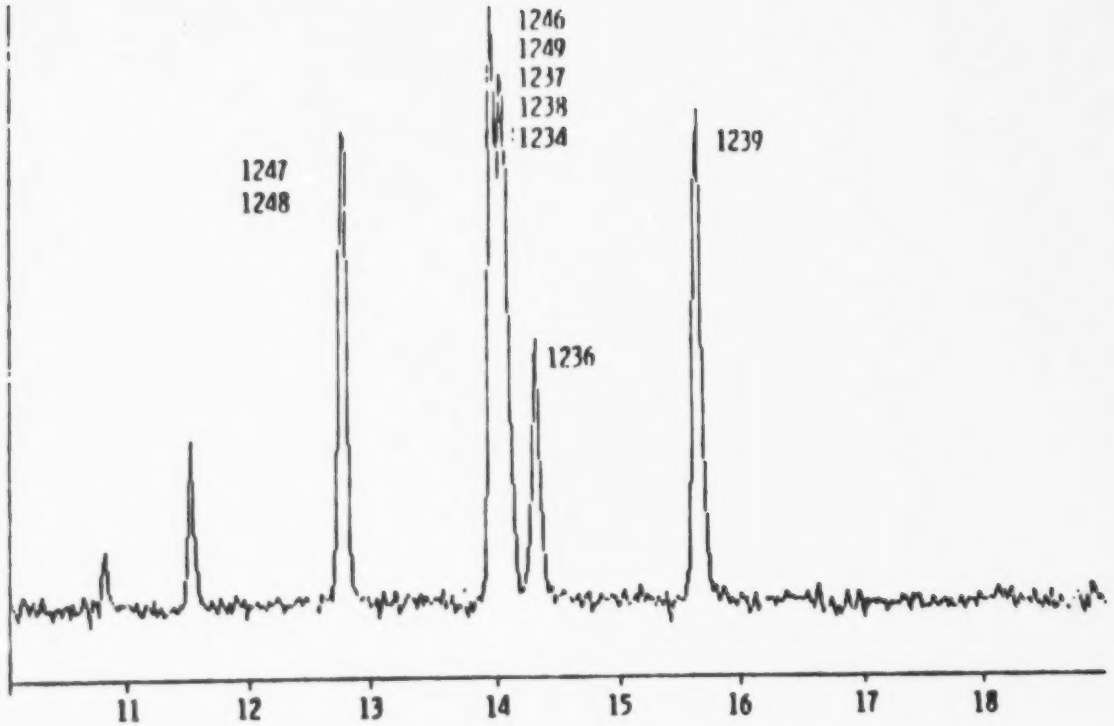


Figure 9. Tetrachlorodibenzodioxin isomers with 3:1 chlorines analyzed on a fused silica SP-2340 column.

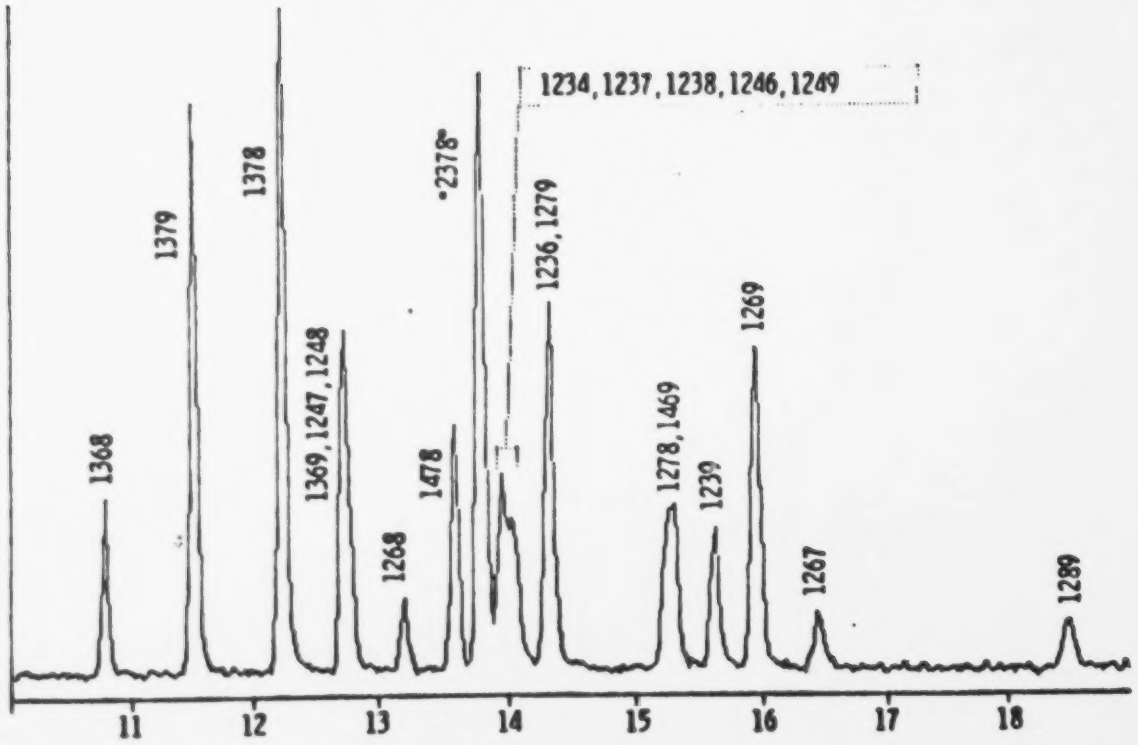


Figure 10. All 22 tetrachlorodibenzodioxin isomers analyzed on a fused silica SP-2340 column.

APPENDIX C

RAW DATA

RAW DATA FOR 2,3,7,8-TCDD ANALYSIS BY WATER TYPE
LOW YOUDEN PAIR, UNITS - NG/L

	DISTILLED WATER		TAP WATER		SURFACE WATER		WASTE WATER 1		WASTE WATER 2		WASTE WATER 3	
AMPUL NO:	5	6	5	6	5	6	5	6	5	6	5	6
TRUE CONC:	26.00	21.00	26.00	21.00	26.00	21.00	26.00	21.00	26.00	21.00	26.00	21.00
LAB NUMBER												
1	22.00	26.00	*	19.00	23.00	25.00	27.00	19.00	23.00	29.00	24.00	17.00
2	18.00	23.00	29.00	20.00	20.00	20.00	30.00	18.00	14.00	15.00	22.00	20.00
3	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*
4	23.00*	25.00*	30.00*	15.00*	32.00*	21.00*	33.00	11.00*	0.00*	0.00*	0.00*	34.00
5	20.20	15.30	18.70	32.10	30.30	19.70	29.70	18.70	18.90	20.70	22.60	24.40
6	31.00	17.00	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	70.00*
7	27.00	24.00	*	26.00	37.00*	31.00*	37.00	33.00*	24.00	22.00	27.00	19.00
8	22.60	16.10	34.60*	22.00*	24.60	21.90	0.00*	0.00*	32.20	0.00*	15.30	16.00
9	21.73	20.30	26.04	20.30	24.19	19.48	25.42	19.27	23.78	19.27	23.78	19.07
10	23.00	22.00	28.00	15.00	31.00	17.00	25.00	18.00	29.00	40.00	19.00	29.00
11	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	20.00	22.00	16.00	25.00	19.00

RAW DATA FOR 2,3,7,8-TCDD ANALYSIS BY WATER TYPE
MEDIUM YOUDEN PAIR, UNITS - NG/L

	DISTILLED WATER		TAP WATER		SURFACE WATER		WASTE WATER 1		WASTE WATER 2		WASTE WATER 3	
AMPUL NO:	3	4	3	4	3	4	3	4	3	4	3	4
TRUE CONC:	81.00	73.00	81.00	73.00	81.00	73.00	81.00	73.00	81.00	73.00	81.00	73.00
LAB NUMBER												
1	65.00	67.00	75.00	75.00	80.00	70.00	72.00	62.00	71.00	63.00	64.00	64.00
2	70.00	58.00	65.00	62.00	65.00	58.00	69.00	64.00	65.00	57.00	66.00	60.00
3	0.00*	0.00*	0.00*	0.00*	105.00*	48.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*
4	168.00*	187.00*	178.00*	159.00*	139.00*	139.00*	186.00*	182.00*	0.00*	0.00*	0.00*	0.00*
5	32.20	49.40	48.30	33.80	62.30	41.60	58.60	77.20	65.10	56.00	61.70	68.50
6	0.00*	92.00	0.00*	115.00	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*
7	82.00	64.00	83.00	63.00	89.00*	72.00*	82.00	76.00	79.00	74.00	56.00	74.00
8	71.60	55.90	81.40*	77.80*	68.40	58.60	0.00*	0.00*	84.30	58.50	68.70	53.40
9	75.03	64.58	78.72	67.45	74.42	63.76	70.32	63.96	77.90	64.58	73.39	63.55
10	55.00	45.00	52.00	49.00	36.00	49.00	68.00	50.00	49.00	52.00	72.00	46.00
11	0.00*	0.00*	0.00*	0.00*	0.00*	82.00	63.00	41.00	70.00	58.00	68.00	55.00

RAW DATA FOR 2,3,7,8-TCDD ANALYSIS BY WATER TYPE
HIGH YOUDEN PAIR, UNITS - NG/L

	DISTILLED WATER		TAP WATER		SURFACE WATER		WASTE WATER 1		WASTE WATER 2		WASTE WATER 3	
AMPUL NO:	1	2	1	2	1	2	1	2	1	2	1	2
TRUE CONC:	202.00	182.00	202.00	182.00	202.00	182.00	202.00	182.00	202.00	182.00	202.00	182.00
LAB NUMBER												
1	178.00	174.00	163.00	175.00	191.00	170.00	181.00	166.00	191.00	207.00	157.00	106.00
2	172.00	214.00	179.00	194.00	177.00	177.00	200.00	189.00	171.00	182.00	183.00	*
3	0.00*	0.00*	69.00*	67.00*	187.00*	220.00*	58.00*	34.00*	167.00*	110.00*	73.00*	36.00*
4	307.00*	228.00*	233.00*	241.00*	312.00*	216.00*	256.00	233.00	0.00*	0.00*	266.00	0.00*
5	130.00	207.00	117.00	112.00	114.00	131.00	116.00	115.00	111.00	73.20	84.50	88.90
6	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	260.00	0.00*	0.00*	0.00*	0.00*	0.00*
7	203.00	168.00	198.00	160.00	218.00*	163.00*	234.00	162.00	179.00	165.00	244.00	146.00
8	204.00	200.00	205.00*	234.00*	214.00	173.00	220.00	291.00	216.00	222.00	158.00	154.00
9	161.13	151.70	169.54	151.70	177.12	151.29	189.63	150.68	178.97	153.14	164.82	151.91
10	140.00	170.00	150.00	160.00	130.00	150.00	140.00	150.00	150.00	170.00	160.00	180.00
11	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	310.00	130.00	160.00	150.00	170.00	130.00